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CEREAL CHEMISTRY

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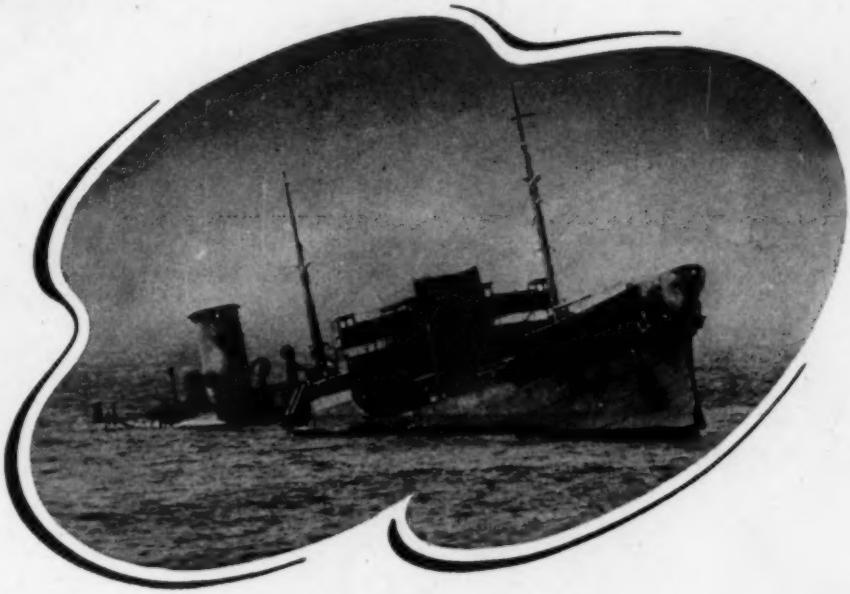

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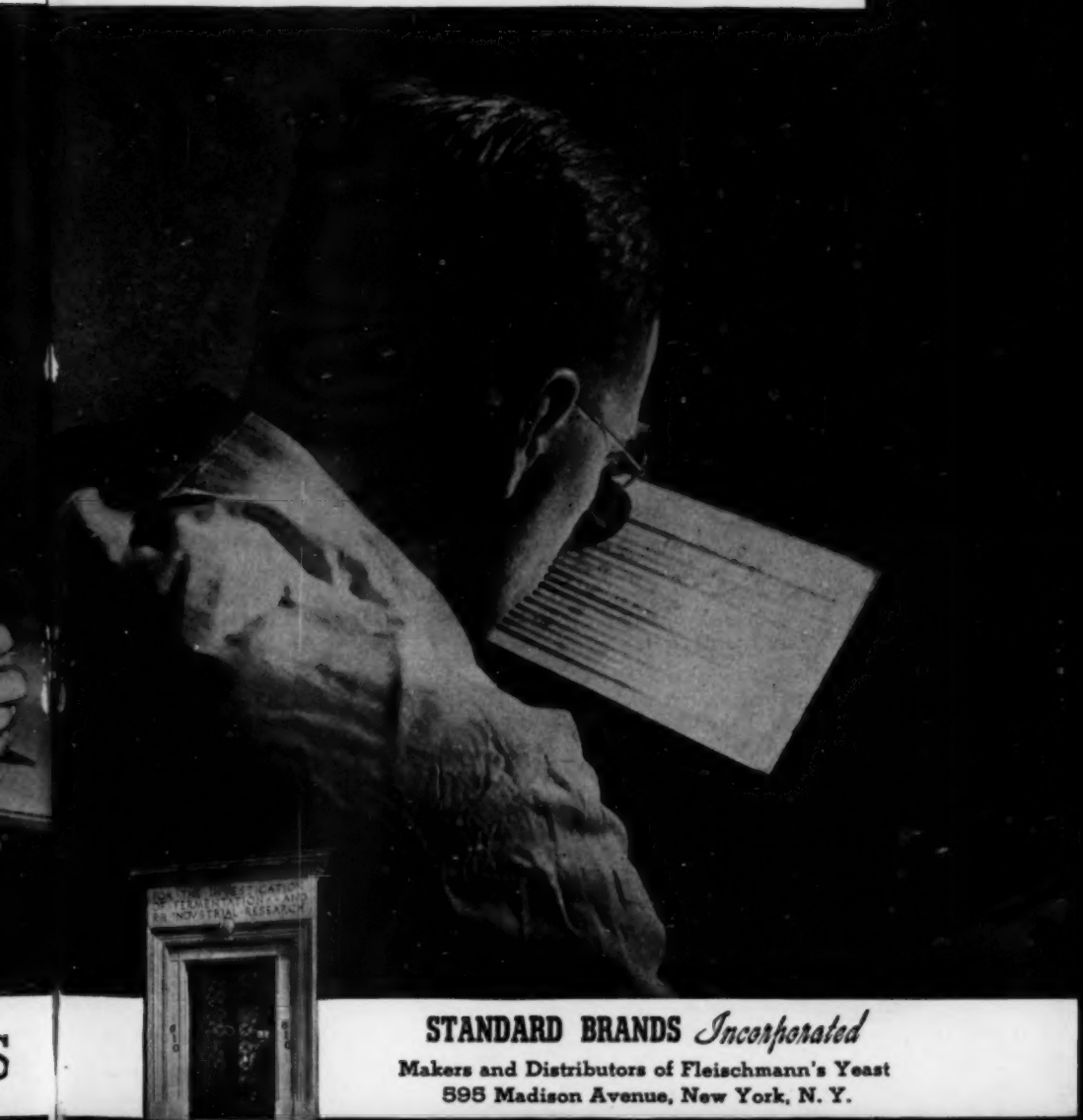

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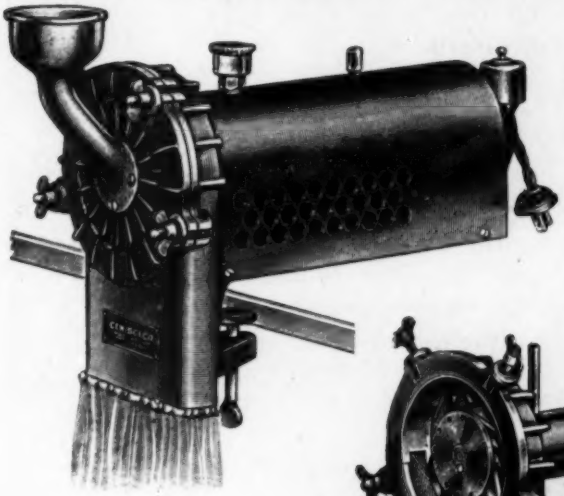

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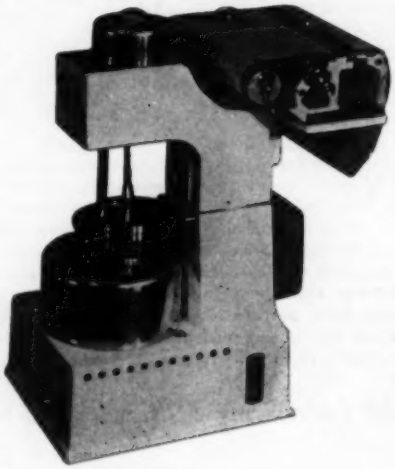

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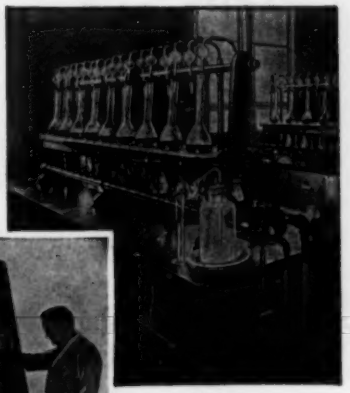

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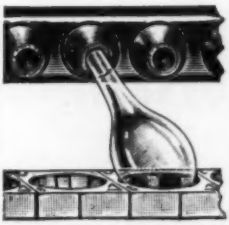

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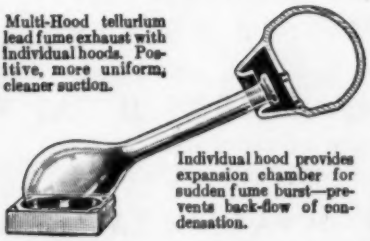

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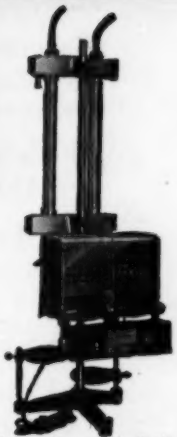

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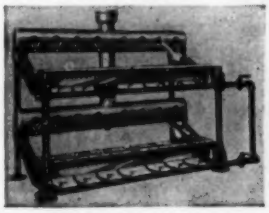

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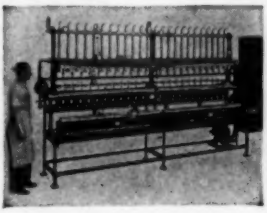

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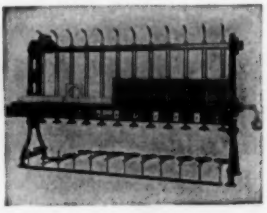

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CEREAL CHEMISTRY

VOL. XIX JULY, 1942 No. 4

INTERPRETATION OF VISCOSITY MEASUREMENTS ON STARCH PASTES [1]

B. BRIMHALL and R. M. HIXON

Iowa Agricultural Experiment Station, Ames, Iowa

(Received for publication March 10, 1942)

The publications on anomalous viscosity [2] and gel properties have been accumulated by technologists working on diversified colloidal products such as tar, paint, plastics, proteins, starches, etc. They are scattered through the various scientific and applied journals and available only in limited scope to the technologists in any one field. The use of particular instruments and the expression of results in specialized form in the different industries is generally a question of habit or convenience rather than of necessity.

A study of this diversified literature was made in search of methods best suited for characterizing starch, the study being a part of the corn breeding program looking forward to the variations in starch properties possible with modern hybridization techniques. This paper attempts to emphasize the connecting thread which runs through this literature and make a knowledge of it available to those interested in its application to starch.

Measurement of Viscosity

The different viscometers and their operation have been adequately described by Bingham (1922), Nivling (1924), Hatschek (1928), Barr (1931), Samec and Blinc (1937), and others. Only two general types are used to any extent for starch pastes:

1. *The capillary type:* This has found widespread use in the laboratory for theoretical studies because it is inexpensive and adaptable. The Ostwald pipette is a familiar example. For routine work on starch it often takes the form of a funnel or a metal cup with a small hole in the bottom, such as the Scott viscometer. Viscosity can be calculated from the rate of flow of the liquid through the capillary or orifice. Ordinarily viscosity is not calculated, but the pastes are com-

[1] Journal Paper No. J-986 of the Iowa Agricultural Experiment Station, Project 426. Supported in part by a grant from the Corn Industries Research Foundation.

[2] An explanation of the various rheological terms is clearly presented by Bingham (1930).

pared on the basis of time required for the flow of a certain volume of liquid.

2. *The rotational type:* Measurements depend on the torque produced on a suspended disk or cylinder as the cup containing the liquid is rotated around it. In some cases the disk is rotated in the stationary liquid. The MacMichael and Stormer viscometers belong to this type. The consistometer (Caesar, 1932; Caesar and Moore, 1935; Radley, 1940) and amylograph are instruments based on the same principle as the rotational viscometer. They are designed for use with very concentrated pastes and give results in which structural viscosity is subordinated to the resistance of the granules to crushing and the thixotropic characteristics of the pastes.

Figure 1 is a diagram of the apparatus used to obtain data for this paper: The pyrex test tube *A* (4.4 cm diameter, 20 cm deep), which

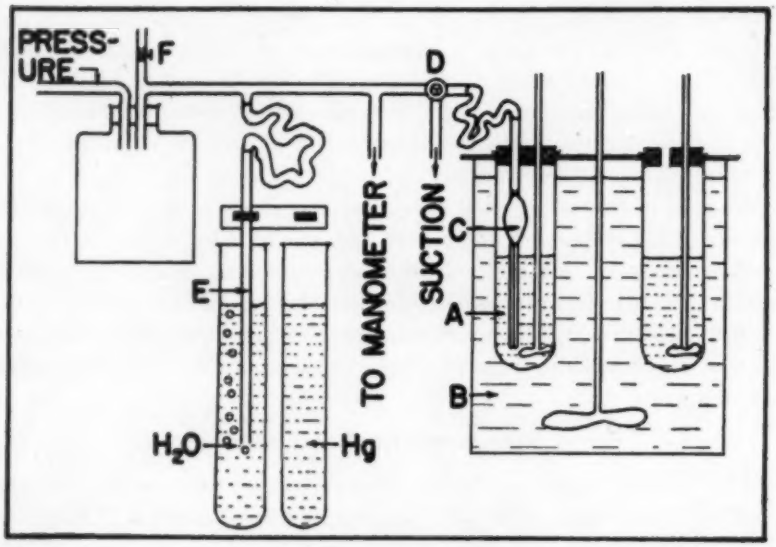

Fig. 1. Apparatus for measuring viscosity.

holds the paste, is immersed in a water bath *B*, the temperature of which is controlled by a thermoregulator. The glass water bath, provided with stirrer and heating coil, is large enough to hold six tubes of paste. The capillary pipette *C* is connected to a three-way stopcock *D* so that suction may be applied to draw the paste up into the capillary or air pressure applied to force the paste down again.

The pressure may be varied by raising or lowering tube *E* in the column of water or mercury surrounding it. A steady stream of bubbles from tube *E* is assured by allowing excess pressure to escape

at F. In this way, pressures up to 300 cm of water can be obtained quickly and accurately. However, it is best to avoid such high rates of shear when possible by using lower concentrations of starch or larger capillaries. The temperature is variable within the range from 25° to 100°C. Measurements are not made above 90° because suction on the capillary causes the paste to become filled with bubbles.

After the water bath has reached the desired temperature, the cold starch-water suspension is poured through the funnel into the viscometer tube, in which a mechanical glass stirrer is rotating at a speed of about 500 rpm. A viscosity-time curve should be obtained for each type of paste in order to find if and when it approaches a relatively constant value for measuring. This enables one to avoid the sharp initial rise and fall from the maximum often observed during the first few minutes of heating (Richardson and Waite, 1933; Briggs and McCarthy, 1942; Katz, Desai, and Seiberlich, 1938). In the apparatus shown in Figure 1, 40 minutes of heating at 90° is sufficient for unmodified cornstarch pastes, although it will vary with concentration of paste, type of container, and the like. After heating the required time, stirring is stopped, the clean capillary introduced, and time of flow determined at various pressures. Viscosity in poises is calculated by Poiseuille's law:

$$\eta = \frac{\pi R^4 g}{8LV} PT \qquad (1)$$

where P = pressure (cm H_2O); T = time of flow (sec); R = radius of capillary; L = length; V = volume; and g = acceleration of gravity.

Eight capillaries ranging from 0.035 to 0.075 cm radius made it possible to measure pastes containing as high as 6% by weight of starch at 90° and higher concentrations at lower temperatures. Experiments using capillaries of six different sizes on one starch paste showed that they can be used interchangeably without appreciable error provided the time of flow is kept in the range between 30 and 200 seconds and there is not too large a spread in capillary dimensions.

The viscosity apparatus described above is similar to that of Richardson and Waite (1933). The reader is referred to their manuscript for an account of the factors affecting viscosity as well as the procedure for preparing and measuring starch pastes. The curves in Figure 2 are typical of the variation in viscosity of a starch paste with rate of shear. Many different theories have been advanced to explain this phenomenon, which is characteristic of systems showing structural viscosity. The experiments of McDowell and Usher (1931) clearly show that the peculiar physical properties of such systems are due to

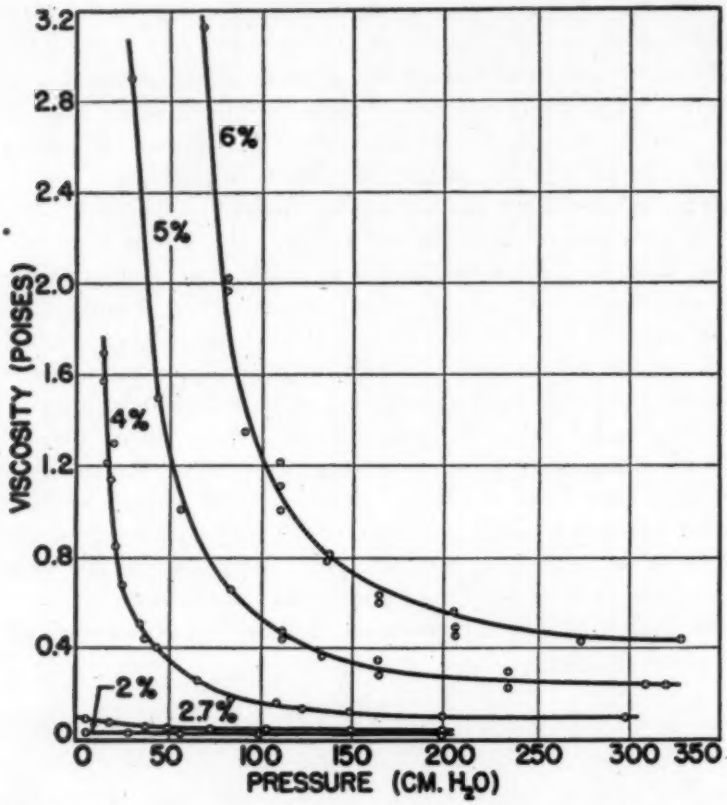

Fig. 2. Viscosity of unmodified cornstarch pastes at 90°C at different pressures and concentrations.

aggregates of particles which surround portions of the solvent and thus increase the effective concentration of solid material. Higher rates of shear tend to disorganize these aggregates to give a more uniformly distributed system. Under the conditions shown in Figure 2, starch suspensions of concentrations below 2% behave as true liquids.

Results on unmodified cornstarch pastes prepared at 90° are plotted in Figure 2 (viscosity-pressure) and Figure 3 (viscosity-concentration). The data at 70° and 80° give similar curves except that structural flow sets in at higher concentrations (7% at 70°; 5% at 80°; 2.7% at 90°). All measurements were carried out on the same batch of starch from one company, which uses somewhat larger amounts of SO_2 in its milling operation than most of the other wet-milling companies.

The following considerations may be applied in general to data from rotation-type viscometers as well as capillary types if it is remembered that rate of rotation corresponds to pressure applied. The

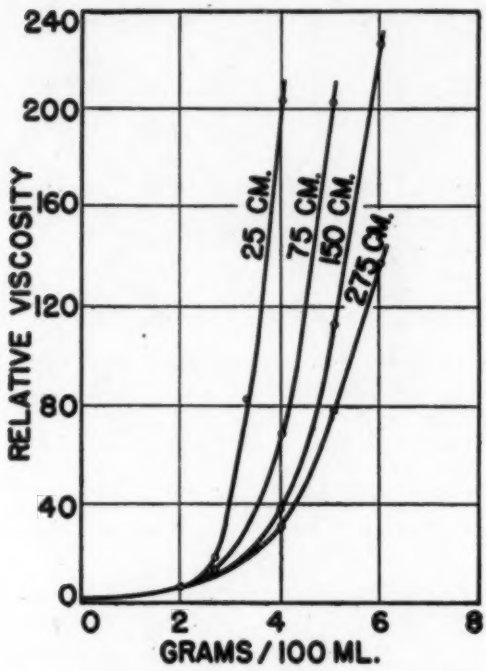

Fig. 3. Viscosity-concentration relationships for unmodified cornstarch pastes at 90°C at different pressures (cm H_2O).

discussion is divided into two parts, one dealing with flow-pressure relationships and the other with viscosity-concentration relationships.

Flow-Pressure Relationships

When rate of flow (V/T) is plotted against pressure, curves like those in Figure 4 are obtained. A straight line proceeding directly from the zero point is characteristic of true liquids such as water; with anomalous liquids like starch pastes, the curves follow the pressure axis for a time before rising and straightening out. Bingham (1922) suggests extrapolating this straight portion back to zero rate of flow (dotted line, Fig. 4) to obtain a "yield shear value," f. Hypothetically this amount of pressure must be applied in overcoming plastic or elastic forces before the paste begins to flow. There have been serious objections to the characterization of starch pastes by yield shear value: (1) Flow actually takes place below the yield value; thus it does not agree with Bingham's definition and has no physical meaning (Farrow, Lowe, and Neale, 1928; Hatschek, 1928). (2) Attempts to characterize modified starches by this method have failed because of the gradual slope of the curves obtained (Gallay, 1936; Porst and Moskowitz,

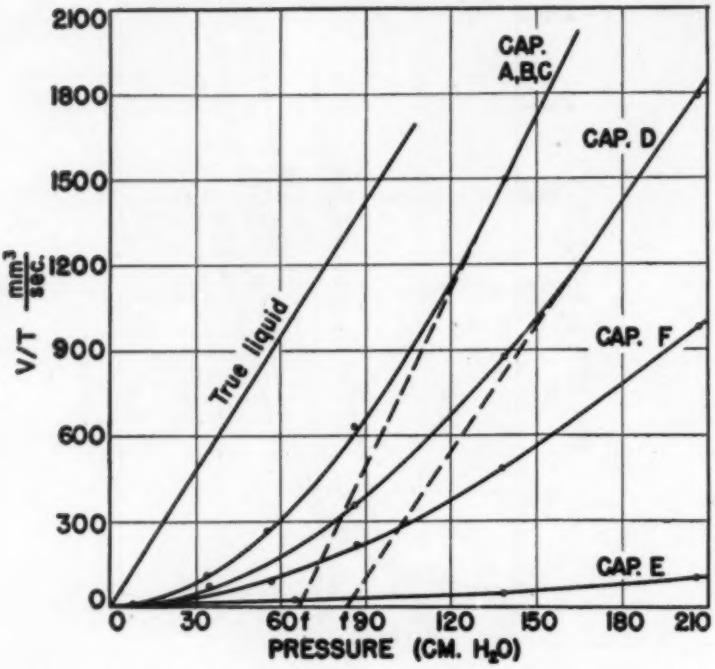

Fig. 4. Variation in rate of flow with pressure for a 4% cornstarch paste at 90°C using different capillary sizes.

1922). Figure 4 shows that extrapolation is entirely too arbitrary in nature, depending upon capillary size as well as the paste.

A slightly different treatment, based on the concept of plug flow, is subject to the same criticism. In this method, the slope of the horizontal portion of the curve, attributed to plug flow through the capillary, is assumed to be in constant ratio to the slope of the vertical portion where plug flow has been replaced by true liquid flow. This ratio has been used to characterize clay pastes (Scott Blair and Crowther, 1929) and flours (St. John, 1927).

A significant advance resulted from the use of logarithmic functions to make straight lines of the troublesome flow-pressure curves. If the logarithm of pressure is plotted against the logarithm of rate of flow (V/T), there are obtained straight lines whose slope is the same for any one paste (within certain limits) regardless of the dimensions of the capillary or type of viscometer. However, the lines will be displaced on the pressure axis according to capillary dimensions. This difficulty was surmounted (Farrow, Lowe, and Neale, 1928; Rabinowitsch, 1929) by splitting the viscosity equation into two parts representing the stress applied to the paste ($RPg/2L$) and the rate of shear

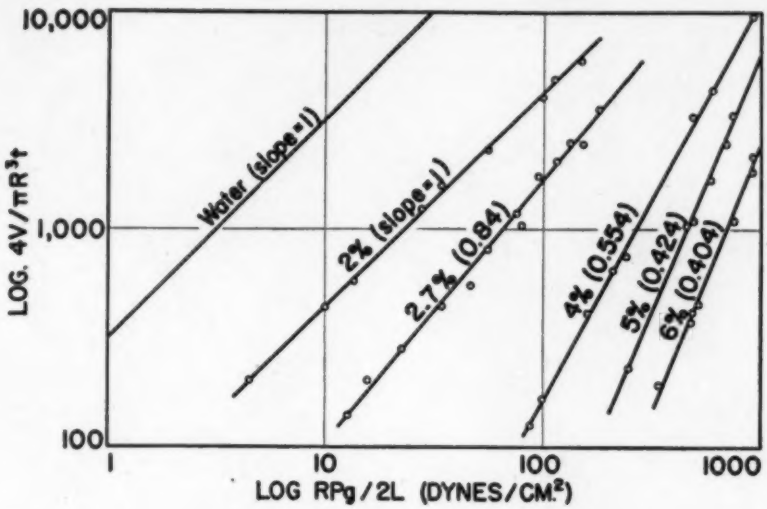

Fig. 5. Shear-stress curves for unmodified cornstarch pastes at 90°C at different concentrations.

produced ($4V/\pi R^3T$). When logarithms of these values are plotted against each other, they give a line whose position is independent of capillary size, its intercept as well as its slope being characteristic of the paste. Rabinowitsch (1929) presents a mathematical justification for this procedure.

When calculated in terms of shear and stress, results from rotation and capillary viscometers may be correlated and expressed by the same constants (Farrow, Lowe, and Neale, 1928). Figure 5 represents shear-stress curves for cornstarch pastes at different concentrations. They fit the equation:

$$\log 4V/\pi R^3T = M \log RPg/2L + \log C \qquad (2)$$

where M is the slope of the line and C the intercept. While the meaning of these constants is not thoroughly understood, it appears that the slope M expresses the degree of structural flow of the paste. For water and a 2% paste, which do not show anomalous viscosity, $M = 1$. Likewise, the intercept C probably is a measure of the comparative amount of stress which must be applied to produce a given rate of shear in the several pastes; the value of C becomes larger as the viscosity of the paste increases.

Table I shows data of this type for a number of different unmodified starches. There is a constant relationship between slope and intercept for any one starch at different concentrations after structural flow has set in, as shown by the relatively constant value of ($I \times S$) in the last

column of the table. Since slope and intercept are so closely bound up with one another, it seems improbable that each can be definitely associated with a distinct physical characteristic of the paste.

TABLE I

INTERCEPT-SLOPE RELATIONSHIP FOR STARCH PASTES

Starch	Concentration %	Intercept [1] I	Slope [1] S	Intercept × slope [2] $(I \times S)$
Commercial I.....	0	−2.42	1.00	0.58
(heated and measured at 90° C)	2.0	−1.62	1.00	1.38
	2.7	−0.98	0.84	1.70
	4.0	+0.76	0.554	2.08
	5.0	+1.40	0.424	1.86
	6.0	+1.64	0.404	1.87
Reid Yellow Dent cornstarch.....	2.15	−1.62	1.00	1.38
(heated and measured at 90°)	2.6	−0.23	0.700	1.94
	3.0	+0.35	0.577	1.93
	4.0	+1.20	0.424	1.78
	5.0	+1.80	0.296	1.42
STARCHES, 2.7% CONCENTRATION, HEATED AT 99°, MEASURED AT 25° C				
Reid Yellow Dent.....		+0.71	0.510	1.86
Commercial Corn I.....		0.53	0.554	1.95
Commercial Corn II.....		0.40	0.625	2.12
Potato.....		1.50	0.554	2.50
Tapioca.....		0.95	0.577	2.28
Rice.....		0.40	0.601	2.04
Waxy Maize.....		0.62	0.625	2.26
Popcorn.....		0.30	0.662	2.18
Mandan White Flour.....		0.56	0.601	2.04
Country Gentleman.....		−0.27	0.753	2.06

[1] These refer to intercept on the $RPg/2L$ axis and slope on the $4V/\pi r^3T$ axis.
[2] A value of + 3.00 was added to all intercept values to make them positive before multiplying.

Viscosity may be calculated from the curves in Figure 5 by the equation:

$$\log \eta = \log RPg/2L - \log 4V/\pi R^3T \qquad (3)$$

since viscosity is equal to the reciprocal of the tangent at any one point.

A set of curves plotted in a similar manner is shown by Gallay and Bell (1936), who obtained data on acid-modified starches using a MacMichael viscometer. The curves were linear within the limits of shear employed, the slope decreasing with increasing modification of the starch. When measurements were carried out over a wide range of shear values, transitions from one type of flow to another (which they designate as structural, laminar, and turbulent) were detected by changes in direction of the logarithmic flow-stress curves. These authors applied the equation:

$$F = KP^n \qquad (4)$$

previously used by Farrow and Lowe (1923), where F = rate of flow, P = pressure, and n is the slope of the line corresponding to M in equation (2). The intercept K, however, contains in it the instrument constants and can be used to characterize the starch only when the same instrument is used in all cases.

Philippoff (1935, 1936) considers that the straight line of the log shear-log stress graph is merely part of an S-shaped curve which has a slope equal to that of water at high rates of shear and curves inward at low rates of shear where proportionately more stress is required. Farrow, Lowe, and Neale (1928) experimentally demonstrated the latter assumption, believing that the elastic properties of the paste become more prominent than its viscous flow at very low rates of shear. This observation is important because it emphasizes the desirability of using gel strength or elasticity measurements to characterize the starch at regions where viscosity determinations fail.

Viscosity-Concentration Relationships

The Einstein equation provided the theoretical foundation for viscosity equations which express concentration in terms of volume occupied by the solid phase. For a colloidal suspension of undeformable spheres he proposed the equation

$$\eta_r = 1 + K\phi \tag{5}$$

where η_r = relative viscosity and ϕ is the volume of spheres per unit volume of suspension. In other words, viscosity is independent of the size of the individual spheres but is a linear function of their total vol-

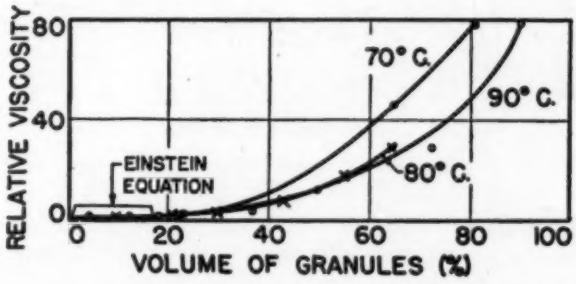

Fig. 6. Relationship between the volume of swollen granules and relative limit viscosity for cornstarch pastes at different temperatures.

ume. The equation is applicable only to the region of the viscosity-ϕ curve where the volume of spheres is relatively small and the curve is linear (Fig. 6). The constant K, representing the slope of the straight-line portion, has been the subject of considerable dispute. Einstein originally evaluated it at 2.5, while Hatschek (1913) believed that 4.5

was more nearly correct. In a specific application to starch, Harrison (1911) found that 4.75 better suited his experimental results. In an attempt to apply Harrison's equation, values of relative viscosity and volume of centrifuged granules found experimentally in this laboratory were used to calculate K. Rather than being constant, it varied all the way from 2 to 6 for unmodified cornstarch pastes. This equation has been discussed further by Humphrey and Hatschek (1916), working with unpasted suspensions of rice starch in carbon tetrachloride, and by Gallay (1936) for acid-modified potato and cornstarch pastes.

For practical purposes this type of equation is not suited for characterizing starch pastes since: (1) It applies only to low concentrations where structural viscosity is lacking. The measurement which is generally desired is the degree of structural viscosity at higher concen-

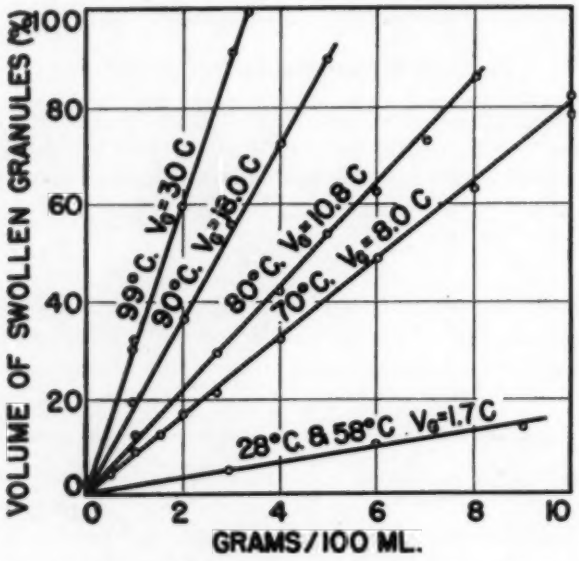

Fig. 7. Relationship between volume of swollen granules (V_g) and concentration of starch (C) at different temperatures.

trations; (2) ordinarily only the weight and not the volume of particles is known accurately (Hatschek, 1928). Starch granules in paste form are seldom undeformable or perfect spheres as the equation demands. This, as well as lack of smoothness and difference in the size of adsorption envelopes on the granules, has been suggested (Hatschek, 1913) to account for the variation in the value of K.

A linear relationship was found between the concentration of starch and the apparent volume of granules at any one temperature of cooking (Fig. 7). The pastes were prepared as described above for viscosity determinations, cooled in running water to room temperature, then

centrifuged in graduated tubes for ½ hour at 1,750 rpm (15 cm radius). The volume of granules is largely a consequence of the temperature used to prepare the paste; thus by using volume rather than weight of starch in calculations, results at different temperatures may be correlated. In Figure 6, relative viscosities at 70°, 80°, and 90°C have been plotted against the apparent volume of granules (V_g) for an unmodified cornstarch. The difference between the curves at 70° and 90° is probably due to a change in the physical state of the granules, such as deformability and tendency to pack in the centrifuge tubes.

Table II summarizes the relative volumes of granules at different temperatures for five starches. The value (V_g/c) is the volume in milliliters occupied by 1 gram of starch in 100 ml of paste after heating at a given temperature and centrifuging.

TABLE II

VOLUME OF CENTRIFUGED GRANULES FOR STARCH PASTES SWOLLEN AT VARIOUS TEMPERATURES

Temp.	V_g/c (%)				
	Wheat	Corn	Rice	Tapioca	Potato
°C	%	%	%	%	%
28	1.6	1.73	1.8	1.73	1.6
49	1.7	1.73	1.86	1.73	1.6
63	3.15	2.38	2.22	15.3	24.0
70	7.0	8.0	5.3	32.0	34.2
80	8.7	10.8	12.0	41.5	42.5
90	12.6	18.0	24.0	33.5	53.2
99	13.3	30.1	28.6	15.3	66.5

The tendency for tapioca granules to become ruptured above 80° is reflected in viscosity and rigidity of tapioca as well as in the decreased volume of granules. This decrease becomes less pronounced if the rate of stirring is lowered during preparation of the pastes, as would be expected.

Another equation involving the volume of granules is that of Hatschek (1913). He believes that at sufficiently high rates of shear, the viscosity becomes constant because the elastic particles become deformed and remain so throughout the flow period, provided they occupy at least 50% of the volume. Under these conditions he proposed the equation:

$$\eta_r = \frac{1}{1 - \sqrt[3]{\phi}} \tag{6}$$

to express relative viscosity where ϕ is the volume of spheres. Sibree (1930, 1931, 1934) tested the equation experimentally on paraffin emulsions and found that the volume ϕ calculated from relative vis-

cosity was in constant ratio (1.3) to the volume of particles determined from photomicrographs of the same emulsions.

In Figure 8, the volume of starch granules determined by centrifuging is compared with the volume calculated from relative viscosity at the same concentration. Although the curves tend to level off at higher concentrations, it can hardly be said that they reach a constant value. Gallay (1936) has discussed this relationship and finds a constant ratio of 0.8 between the volume of granules (calculated from viscosity at the critical concentration) and the reciprocal of the critical concentration (where he believes the granules affect the whole volume of solution) for a series of acid-modified corn and potato starch pastes.

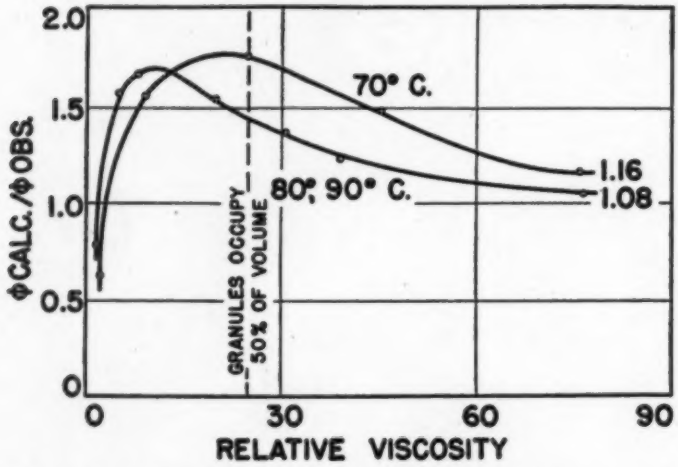

Fig. 8. Ratio of volume of granules (ϕ) observed by centrifuging to that calculated from viscosity by the Hatschek-Sibree equation.

Mark and Simha (1940) have recently reviewed the progress made in the study of nonspherical particles of high polymers with respect to their viscosity and molecular structure.

Logarithmic expressions are sometimes used for viscosity-concentration relationships. Rask and Alsberg (1924) compared a series of wheat starches by plotting the logarithm of viscosity against concentration to give a straight line with the equation:

$$\log y = \log b + mx \tag{7}$$

where y = viscosity of the suspension and x = concentration of starch. Each starch was characterized by the constant m, the slope of the line, and $\log b$, the intercept on the concentration axis, which is frequently the viscosity of the solvent itself. This type of equation, proposed originally by Arrhenius in the form:

$$\eta_s/\eta_0 = C \log A, \tag{8}$$

has since been applied to various colloidal systems. For example, Traxler, Schweyer, and Moffatt (1937), using the exponential form:

$$\eta = C10^{AF}, \tag{9}$$

found that it fitted their viscosity data on asphalt-mineral powder suspensions.

In Figure 9, viscosity-concentration data for unmodified cornstarch pastes prepared at 90°, 80°, and 70° are treated according to the method described above. The fan-shaped group of curves shows that the method must be used with caution. In this case straight lines are

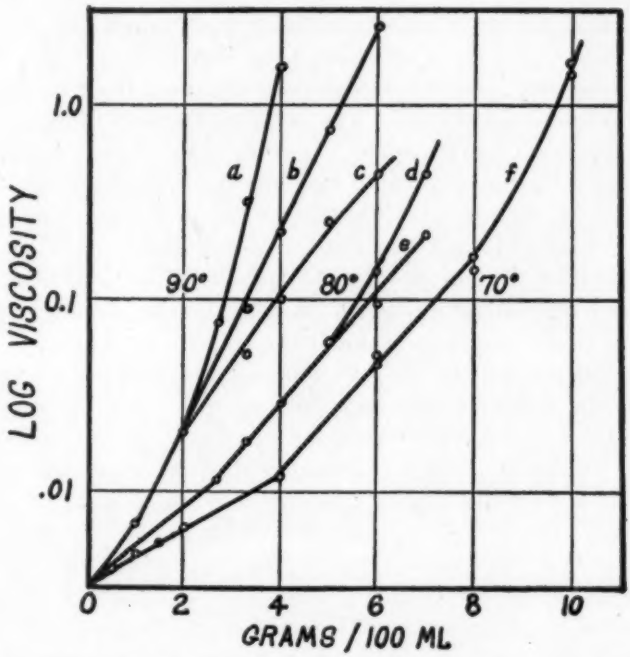

Fig. 9. Application of the Raak-Alsberg method to starch pastes at different temperatures and pressures: *a*, 15 cm H_2O pressure; *b*, 75 cm; *c*, 275 cm; *d*, 44 cm; *e*, 140 cm; *f*, 85 and 135 cm.

obtained only at certain ranges of concentration and pressure. At low pressures, the logarithm of viscosity increases more rapidly than the concentration, giving curves convex to the viscosity axis. This tendency is even more pronounced at 70° than at 90°. Morgan and Vaughn (1942) report this effect with slightly dextrinized starch mixtures. Similar curves for waxy maize starch pastes were inclined to slope in the opposite direction, perhaps because of a crowding and breaking effect on the more susceptible granules as concentration and rate of shear increased.

Discussion

Industrial control of starch products is generally based on the measurement of flow properties, the fluidity funnel being the commonest type of instrument. A comparison of the latter with rotational and capillary type viscometers has been made by Morgan and Vaughn (1942) and Gallay and Bell (1936). Despite the widespread use of viscosity methods, many starch workers are under the impression that such measurements cannot be made on starch pastes with any degree of precision. It is hoped that this idea will be dispelled by the evidence which is accumulating against it, particularly in the publications of the British Textile Institute. Richardson and Waite (1933) state that if the conditions of flow are standardized either by specifying the shearing stress or the rate of shear at which a measurement is made, the same value of apparent viscosity of a paste is obtained from measurements made in different capillaries. The work of Farrow, Lowe, and Neale (1928) is especially convincing in that flow values from both rotational and capillary viscometers could be plotted on the same straight line (log shear—log stress). Duplication of results by different operators using the fluidity funnel has also been demonstrated (Morgan and Vaughn, 1942). But more important than the method of measuring flow is accurate control during preparation of the paste—it should be allowed to attain equilibrium under the conditions chosen.

Quite independent of the inherent composition of the starch, such factors as the following influence the apparent viscosity of its pastes: (1) aggregates of granules which surround portions of the liquid medium and behave as a unit; (2) granules which have swollen sufficiently to become deformable under pressure and no longer act like rigid spheres; (3) empty sacs of granules which have collapsed; and (4) soluble material which has diffused out of the granules and changed the composition of the dispersing medium. It is apparent that no one mathematical expression could be expected to account for the response of each of these factors to changing pressure, temperature, time, concentration, and the mechanical factors operating during preparation of the colloidal system.

There have been proposed a number of viscosity methods which involve the presence of added reagents. Some of them attempt to subordinate the gross colloidal factors of the system to the factors more inherent to the starch itself. A recent example is "thiocyanate viscosity" (Richardson, 1939; Jambuserwala, 1941) in which the starch is homogenized and dispersed in calcium thiocyanate. In other methods, the added reagent is intended only to stabilize the viscosity, such as formaldehyde (Farrow, Lowe, and Neale, 1928). The soap-starch viscosity procedure (Houtz, 1941; Heald, 1941) supposedly augments

structural viscosity under the proper conditions and is reported to give a good indication of strength which the starch will impart when used as a paper size.

When it comes to a study of the starch itself, such methods all have the disadvantage that another variable (added reagent), whose effect is not thoroughly understood, is added to an already complex system of many variables.

Hot viscosities have been used throughout this paper in order to avoid the added complications introduced by cooling such systems. Although a clear picture of what happens when a starch paste is cooled cannot be given from the present state of knowledge, the authors' concept is stated as follows:

When a starch paste is cooled, portions of it tend to revert to a more insoluble form, a phenomenon often included under the terms *aging*, *retrogradation*, or *crystallization*. It is accompanied by an increase in opacity of the paste. If retrogradation is accelerated by cooling or freezing, the retrograded starch can be shown by X-ray diffraction measurements (Bear and French, 1941) to be at least in part crystalline. Starches differ markedly in their tendency to retrograde, waxy maize being especially resistant to crystallization. Within a starch itself, one fraction (amylose) retrogrades easily, in contrast to the amylopectin fraction. This difference in behavior may be associated with the straight-chain or branched structure of the molecule.

It seems logical to explain the nonfluid condition of starch gels as caused by an interlacing of crystalline starch fragments between the aggregated starch granules as well as within the granules themselves. It has been shown that starch gels of sufficiently high concentration are true solids rather than colloidal liquids and exhibit true elasticity (Hixon and Brimhall, 1941).

The preceding paragraphs carry the implication that viscosities should be measured on hot pastes rather than on cold pastes to avoid the variations in crystallizing tendency shown by different starches and starch fractions. The suggestion might be made that the effect of cooling is not merely to increase the degree of structural viscosity by causing aggregation, but that it causes a change in the physical character of the starch itself (crystallization), which in high enough concentrations results in its assuming a solid, elastic character. The degree of elasticity may be used to characterize the cooled gel and measured as a property distinct from viscosity (Brimhall and Hixon, 1939). A clearcut illustration of this is provided by a comparison of the rigidity and viscosity of waxy maize starch with that of ordinary cornstarch, the former having very high hot viscosity but almost no tendency to set to a gel.

Summary

The various methods for interpreting viscosity measurements as applied to starch pastes are reviewed. The application of these methods is illustrated with data obtained by the authors at different temperatures, concentrations, and pressures, with a capillary viscometer.

It is pointed out that starch paste is a heterogeneous system, each constituent of which may react differently toward changes in the conditions of measuring viscosity. The extreme susceptibility of the colloidal state of starch to variations in the method of preparation is stressed. These induced changes are such that no one mathematical expression could be expected to represent flow characteristics over a very wide range.

Measurement of hot viscosity rather than cold viscosity is recommended to avoid the additional complications introduced by cooling the paste. The quantitative determination of gel strength or elasticity as a measure of the solid characteristics of a starch is considered to be a valuable adjunct to viscosity, which is a measure of its fluid characteristics.

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DEXTRINS: THE RELATION OF FERMENTABILITY TO SOLUBILITY AND REDUCING POWER

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The starch factor in flour and bread has recently received much attention from cereal chemists. The susceptibility of starch to the attack of amylolytic enzymes is a problem the solution of which is fundamental in appreciating the complicated role which starch plays in a flour-water-yeast system.

Raw starch cannot be used by yeast in the manufacture of carbon dioxide gas. It must first be placed in proper condition for conversion to sugar by enzymes normally found in flour. Now it has been found that starch which has been damaged, by milling for example, is more readily attacked by these amylolytic enzymes. Apparently, milling produces starch-breakdown products, which, being of a lower order of complexity, lend themselves more readily to further reduction by enzymes. These changes in susceptibility have been measured by Newton, Farley, and Naylor (1940).

Familiar among the products of starch decomposition are the dextrins. They are those interesting carbohydrate compounds, ranging between raw starch and sugar, which are as yet relatively unexplored. Because of their complex nature little is known of their structure. Their behavior in dough fermentation should largely depend upon their rate and degree of conversion to fermentable sugars by the flour amylases. Kent-Jones and Amos (1940) showed that the quantity of dextrins in bread dough is a factor in the handling and baking of such a dough, and that this factor influences the character of the crumb in the finished bread. These workers devised a quantitative test for dextrin in flour.

There is, however, another factor worthy of consideration and that is the character of the dextrin. This investigation has been conducted on commercially available dextrins to demonstrate that these substances vary greatly in composition and in effect on fermentation.

Commercial dextrins are manufactured by heating pure starch in the presence of small quantities of mineral acids. The chemical make-up of these dextrins changes continuously under this treatment. It is possible therefore to get a host of different mixtures of these compounds known as dextrins, merely by varying the heating time and/or the acid (Bauer, 1912). For example, it has been known for some time that the percentage of soluble material in the dextrin varies with the

treatment time and the amount of acid used. This has been noted by Schoch (1941). In commercial practice, this figure is often used as a measure of dextrinization. The reducing values of dextrins also change with the type of treatment.

In this paper we shall attempt to show the relationship in dextrins of fermentability to reducing value, on the one hand, and to solubility on the other.

Methods

In this investigation ordinary flour-water-yeast doughs were employed to demonstrate fermentability. The dextrins were added to these doughs by dry-mixing them with the flour. Aliquot portions of these doughs were carefully weighed off and placed into a pressure-meter apparatus similar in principle to, but differing in design from, that employed by Sandstedt and Blish (1934) described by Glabe (1942, Fig. 1). The amount of gas evolved was recorded at hourly intervals up to 4 hours and then again at 4½ hours. The values obtained were then calculated to the base of 100 g of flour at 15% moisture at 760 mm of pressure and 30°C. The figures obtained at the 4½-hour period were used for comparing fermentabilities.

Solubility was determined by stirring a given quantity of dextrin in water, filtering, and determining the specific gravity of the filtrate. The specific gravity gives a measure of the amount of soluble material in the dextrin. Reducing values were determined by the Schoorl volumetric method.

Increase in Gas Production by Dextrin

In the first step the relationship of the reducing value of the dextrin to its gas-producing ability was determined. A popular short patent flour which had been lightly malted and bleached was used as a substrate. The effect upon gas production of adding to this flour 1%, 2%, and 3% of a commercial corn dextrin was compared with the effect produced by adding similar quantities of cp dextrose, maltose, and sucrose. The results are shown in Table I. The dextrin upon analysis showed a reducing value of 7.93% calculated as dextrose. For all three percentages the dextrin, because of its amylase susceptibility, showed a larger increase in gas over that produced by the control than would have been expected from its reducing value.

A second series of tests with the same flour showed that whereas 3% of dextrose added to the flour produced 2,225 ml of gas per 100 g of flour, and 3% of dextrin produced 1,890 ml of CO_2, a quantity of dextrose equivalent to the reducing value of 3% of the dextrin produced only 1,620 ml. The flour with no additions of dextrose or

TABLE I

DEXTRIN SERIES I—EFFECT ON GAS PRODUCTION OF ADDITIONS OF COMMERCIAL CORN DEXTRIN TO DOUGH AS COMPARED TO ADDITIONS OF EQUAL QUANTITIES OF DEXTROSE, SUCROSE, AND MALTOSE

Substance added	CO_2 produced in 4½ hours by 100 g flour		
	1%	2%	3%
	ml	*ml*	*ml*
Dextrin	1,635	1,735	1,890
Dextrose	1,825	2,030	2,225
Sucrose	1,905	2,065	2,210
Maltose	1,825	2,020	2,225
Gas production of the flour	1,550 ml		

dextrin showed a level of 1,560 ml. This again is attributable to saccharification of the dextrin by flour amylases.

Since solubility, as mentioned before, is used as a measure of dextrinization in commercial practice, this factor might be found to bear some relationship to differences in fermentability.

Solubility vs. Gas Production

A series of three commercial corn dextrins of varying solubilities was studied in an attempt to correlate solubility with fermentability. This is dextrin series II.

In this series of tests two hard winter wheat flours were employed. Both were of the same extraction, but flour A was unbleached and unmalted, and B was unmalted but bleached. The maltose value for flour A was 275 and for flour B 268. To these flours were added 0.1% of commercial malted wheat flour in conjunction with varying quantities of the dextrins.

Table II gives the characteristics of the dextrins of series II and the results for total gas produced by adding 3% of the dextrins to unbleached flours A and B plus 0.1% of malted wheat flour. It will be observed that although the solubility of dextrin No. 3 was greater than that of No. 1 and No. 2 it produced less gas than either. The reducing value of dextrin No. 3 was also slightly greater than that of No. 2, yet the gas produced by dextrin No. 3 was less than that produced by No. 2. Apparently this slight advance in reducing value is of no consequence in the light of relative gas-production values.

Relatively similar results were obtained when the bleached flour B was used, although the amount of gas produced by each dextrin was slightly less than when unbleached flour was used. The values seem to indicate a relationship of some sort between "degree of dextrinization" and fermentability or condition for saccharification by enzymes.

TABLE II

DEXTRIN SERIES II—EFFECT ON HARD WHEAT FLOUR OF ADDITIONS OF 0.1% OF MALTED WHEAT FLOUR AND 3% OF COMMERCIAL CORN DEXTRINS OF VARYING SOLUBILITY

Dextrin No.	Soluble material	Reducing value as % dextrose	CO_2 produced in 4½ hours by 100 g flour	
			Unbleached flour A	Bleached flour B
	%	%	*ml*	*ml*
1	30	4.12	1,980	1,910
2	60	8.03	2,060	1,975
3	80	8.28	1,860	1,855
Flour gas production	—	—	1,730	1,690
Flour plus malt flour	0.1	—	1,775	1,750

In order to explore this idea, commercial dextrin runs were made in which 1400 lbs of corn starch was heated at 265°–270°F in the presence of 3500 ml of hydrochloric acid at 10 Bé with constant agitation. Samples were drawn off at regular intervals during a four-hour treatment in order to obtain a set of dextrins varying in solubility from low to extremely high percentages. These dextrins comprise group III.

Samples were tested for solubility, reducing power, and fermentability at 3% increments as previously. The upper curves of Figure 1 show fermentability or gas-production values plotted against solubility of the dextrins of series III. The solid line represents the total gas produced with unbleached flour; the dotted line gives the results with bleached flour. The lower curve represents reducing power (as percent of dextrose) plotted against solubility. It will be noted that the first appreciable increase in gas production over the control was found when the solubility of the dextrin reached 4.0%. Fermentability increased up to the solubility of 69%. Thereafter fermentability decreased as the solubility rose. It is immediately apparent that the gas-production values of this series of corn dextrins are more a function of the solubility than of reducing power, since gas production begins to fall even though reducing values continue to increase.

The extreme right-hand portion of the fermentability curve also shows an interesting characteristic. The last three dextrin samples which were collected had solubilities of 100%. The last two dextrins apparently had a singular effect on fermentation, since the gas-production values as indicated by the curve were less than the value of the control, and this in the face of the fact that they showed a reducing value of more than 5%. The curve of the bleached flour shows a

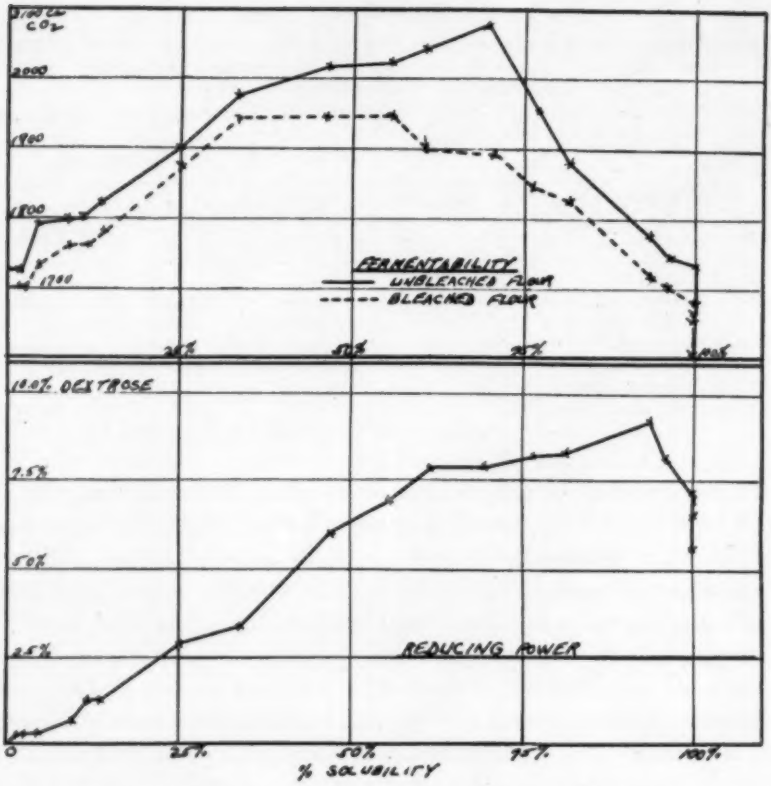

Fig. 1. Dextrin series III—fermentability vs. solubility and reducing power.

very interesting point in that the highest fermentability is reached at 56% solubility as against the highest fermentability with the unbleached flour of 69%.

Summary

Several series of dextrins made from acid-hydrolyzed corn starch yielded increases in gas production of fermenting doughs not always compatible with their reducing-power values. These corn dextrins increased in fermentability and susceptibility to amylase attack as their solubility in water rose to 69%, thereafter suffering a rapid decline. At the ideal solubility the amount of gas increase produced by these corn dextrins was many times that which would be produced by a quantity of dextrose of equivalent reducing value, indicating that this is the point of maximum amylolytic conversion.

There were some indications that the dextrinization of corn starch, when continued until the starch was 100% water soluble, produced material which was not fermentable, even after exposure to amylases.

Reducing power in dextrins is not always a true indication, either of the extent of dextrinization or of fermentability.

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THE NATURE OF THE INTERFERING CHROMOGENS ENCOUNTERED IN THE DETERMINATION OF NICOTINIC ACID IN CEREAL PRODUCTS

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Since the establishment of the importance of nicotinic acid and nicotinic acid amide in nutrition, a number of investigators have reported work carried out on various materials. The primary object of these investigations was to determine the nicotinic acid content of the specific material in which the investigator was interested, and hence to develop a suitable procedure applicable to the specific material. Most of the material investigated was of animal origin and the problems encountered were usually specific for this type of product. As a result several procedures have shown sufficient promise in the work to which they were applied to suggest their use to workers interested in the nicotinic acid content of other products. When these different procedures are used for the estimation of the nicotinic acid content of any one product, the results obtained, as a rule, are different. Most of the investigators recognize that their procedures are limited to specific material and are not of general application.

The varying results reported for the amounts of nicotinic acid in cereal products by the application of different procedures has caused

confusion in regard to the actual nicotinic acid content of cereals. At the government hearing on bread standards in Washington, data were supplied on the nicotinic acid content of wheat, various grades of flour, and bread. These results were two to three times higher than the amounts of nicotinic acid that we have been able to find in comparable grades of flour and bread. They were also not reconcilable with the fact that such materials are low in nicotinic acid when tested by animal experimentation for black tongue. The chemical methods by which these results were obtained were not stated.

Bina, Thomas, and Brown (1941) developed a procedure for the determination of nicotinic acid in cereal products that gave consistent results on all products to which it was applied. Thomas, Bina, and Brown (1942) applied this method to yeast, bread, and other cereal products and showed that wheat and flour do not contain the high amounts of nicotinic acid that are indicated by some of the other procedures, but showed amounts in line with the fact that these substances are low in nicotinic acid. These authors also pointed out that interfering substances that produce high values are likely to be present in the extracts from cereal products, and that the procedure must be designed to avoid such interferences. Since the procedure of Bina, Thomas, and Brown was designed to eliminate this interference, it did not include any discussion of the nature of the interfering substances.

All of the procedures suggested for the determination of nicotinic acid are based on the method of König, in which cyanogen bromide is used to react with the nicotinic acid, and the reaction product in the presence of a suitable aromatic amine combines to produce a color reaction that can be measured. The color developed is compared to the color produced with a known amount of nicotinic acid treated similarly. The procedures differ in the preparation of the extract, the method selected for making the extract preparatory to color development, the aromatic amine used, and the technique employed in the color development.

All of these steps are important and it will be shown in the experimental part of this paper that an interfering substance is produced causing abnormally high results in cereal products when determined by the procedure of Melnick and Field (1940). We find that this interfering substance is developed, in the hydrolysis step of their procedure, by the treatment with concentrated hydrochloric acid and is not removed by their subsequent treatment. The decolorizing technique with charcoal which these authors employed for decolorizing their extract has no effect on the removal of this substance, and it remains in the solution to give interference by their procedure of color development with cyanogen bromide and aniline. The fact that the

color produced by this compound, cyanogen bromide, and *p*-aminoacetophenone is not extractable with ethyl acetate shows the importance of the ethyl acetate step in the procedures that take advantage of this selective solvent to extract the color produced by these reagents in the nicotinic acid determination. Furthermore, it is shown that when this interfering substance is removed or avoided in the preparation of the extract the nicotinic acid content of cereal products becomes identical with the results obtained by Thomas, Bina, and Brown (1942).

A further point of interest is the fact that this interfering substance is not encountered in the determination of nicotinic acid in yeast products by the use of concentrated hydrochloric acid in the hydrolysis.

Waisman and Elvehjem (1941), using essentially the procedure of Melnick and Field (1940), report a value obtained on yellow corn of 10.7 mg per 100 g or 48.47 mg per pound. Since this material was used in their routine production of black tongue in dogs, they concluded that the presence of some unknown chromogen other than nicotinic acid was responsible for the high value and that this substance does not posses antipellagric properties.

Dann and Handler (1941) report values for nicotinic acid in samples of yellow corn which are in close agreement with results we obtain. They found 6 to 10 μg per gram on these samples, corresponding to 2.7 to 4.5 mg per pound. This compares with 3.17 mg per pound on the sample we analyzed. These authors remove the nicotinic acid from the extract (and thereby free it from the compounds not removed) by Lloyd's reagent. Since Lloyd's reagent also removes all of the alkaloidal substances and coloring material present in the extract along with the nicotinic acid, they further treat with lead nitrate the colored solution produced on dissolving the Lloyd's precipitate in caustic. This produces a colorless solution containing the nicotinic acid which these authors deem essential for their color development with metol.

Kodicek (1940) reports values for nicotinic acid obtained on yellow corn ranging from 6 to 27 μg per gram, dependent upon whether the determination was made on the aqueous extract subsequently hydrolized with sodium hydroxide, or the extraction was made originally with 8% of sodium hydroxide solution. This difference was not due in his opinion to imperfect extraction but to some unspecific chromogen which was not in fact a pellagra-preventing substance. This author tested this corn by feeding to dogs and found they all developed black tongue in the usual period of time. From the negative biological results obtained he concluded that the water extract which showed 6 μg per gram provided the true value for this substance and that extraction with boiling water permits the quantitative extraction of active nico-

tinic acid. Similarly, wheat germ gave considerably higher values when the extraction was made with 8% sodium hydroxide (39 μg per gram) than with boiling water (27 μg per gram). It is to be observed that Kodicek used *p*-aminoacetophenone in this work for the color development. He did not employ ethyl acetate to extract the color complex developed where interfering substances are present, as recommended by Harris and Raymond (1939).

Experimental

In order to establish the cause of the wide differences in results obtained on various cereal products by different chemical procedures of analysis for nicotinic acid, experiments were planned to include determination of the nicotinic acid content of the same materials by different procedures.

Yeast: A sample of regular bakers' yeast was used for the first set of experiments. The nicotinic acid content of this material was determined by the procedure of Bina, Thomas, and Brown (1941) and of Melnick, Oser, and Siegel (1941). The results obtained on this yeast gave 0.391 mg of nicotinic acid per gram by the first procedure and 0.394 mg per gram by the second procedure.

An enriched yeast was next used for the determination by the two procedures. 515.0 mg per pound was obtained by the Bina, Thomas, and Brown procedure and 520.4 mg per pound by the Melnick, Oser, and Siegel procedure. These results are practically identical and show that no substance was encountered that affected the determination of nicotinic acid in yeast by either procedure. The values are also in line with the amounts of nicotinic acid that are expected to be present in the two samples of yeast.

Whole-wheat flour: Whole-wheat flour was used for this set of experiments and nicotinic acid was determined by the same procedures as with yeast. The results obtained, however, were entirely different by the two methods, as this flour showed 7.44 mg of nicotinic acid per pound by the Bina, Thomas, and Brown procedure and 21.47 mg per pound by the Melnick, Oser, and Siegel procedure.

Since the extract prepared by the latter took on considerable color in the treatment and contained alcohol, the remaining solution, from which the above aliquot was taken, was evaporated to dryness on the water bath, treated with 15 ml of concentrated nitric acid, and again carried to dryness. This treatment with nitric acid was repeated twice more to oxidize as much of the organic material as possible before finally diluting with water and evaporating to remove the nitric acid. The residue was then made up to volume with water and alcohol for further analysis. One aliquot of this retreated extract was used for

the determination of nicotinic acid by the Melnick, Oser, and Siegel method. The results show that the blank value had been reduced considerably but the apparent nicotinic acid value of the sample had been slightly increased by the treatment. A value of 22.98 mg per pound was obtained on this retreated extract as compared to 21.47 originally determined.

A second aliquot of this solution was used for the determination of nicotinic acid by the Bina, Thomas, and Brown procedure. This method employs *p*-aminoacetophenone instead of aniline in the color development, and the color complex produced is extracted with ethyl acetate so that the readings can be made on the ethyl acetate solvent instead of the original extract as is the case in the Melnick, Oser, and Siegel procedure. The results showed 8.29 mg per pound on this extract against 7.44 mg per pound as determined on the original material by this method. It is apparent that the nitric acid treatment did not remove any of the color affecting the determination even though the blank values were considerably reduced. It is also evident that this extract contains a large amount of material other than nicotinic acid that produces a color complex with aniline, and consequently is determined as nicotinic acid by the Melnick, Oser, and Siegel procedure. This color complex is not extractable with ethyl acetate in the Bina, Thomas, and Brown procedure and, therefore, is eliminated from the nicotinic acid measurement by that method.

Dann and Handler method: A sample of the same whole-wheat flour was subjected to hydrolysis and treatment according to the procedure of Dann and Handler (1941) for the determination of nicotinic acid. In this procedure the acid hydrolysis is made on the sample instead of on the aqueous solution, with a relatively high concentration of hydrochloric acid and at boiling-water-bath temperature for two hours. The extract was next treated with Lloyd's reagent to adsorb the nicotinic acid and the precipitate was washed with 0.2 *N* sulfuric acid and redissolved in caustic so that it could be decolorized with lead nitrate according to prescribed procedure. A water-clear, practically colorless solution was obtained. This solution was then treated with cyanogen bromide and metol according to the color production technique of this procedure. The color produced, however, was dark brown instead of the characteristic lemon yellow, and therefore not readable in the fluorophotometer.

Another sample was hydrolyzed and treated by the same procedure up to the point of color development. One aliquot of this solution was treated with aniline and cyanogen bromide according to Melnick, Oser, and Siegel and the other with *p*-aminoacetophenone according to Bina, Thomas, and Brown's color technique. The results showed

22.52 mg per pound by the Melnick, Oser, and Siegel method and 8.13 by the Bina, Thomas, and Brown procedure. These results, where the procedures used for extracting and hydrolyzing the samples differed, are not materially different from the preceding results where the procedures used were in accordance with the authors' recommendations.

Action of oxidizing agent on the interfering chromogen: 20 g of the same whole-wheat flour as previously used was hydrolyzed according to the procedure of Dann and Handler, with relatively concentrated hydrochloric acid. This extract was made up to a known volume and aliquots used for further treatment. This extract was highly colored and contained considerable chromogen, other than nicotinic acid, that produces color with cyanogen bromide and aniline according to the procedure of Melnick and co-workers.

Ten ml of this solution was treated with 5 ml of strong hydrogen peroxide by placing both in a test tube and heating in a water bath at 60°–70°C as long as oxygen was given off. The water-bath temperature was then brought to the boiling point for a half hour to remove any excess hydrogen peroxide from the acid solution. The hydrogen peroxide decolorizes the solution to give a water-clear extract. This solution was neutralized and the nicotinic acid determined with aniline according to the Melnick, Oser, and Siegel procedure, the results being compared to the original extract. The apparent nicotinic acid content was reduced from 23 mg per pound on the original extract to 13 mg per pound by this treatment.

Another 10-ml portion of the extract was treated with hydrogen peroxide to give a colorless extract as above, and then concentrated to dryness on the steam bath to expell the excess hydrochloric acid. The residue was dissolved in water and gave a strongly colored solution as a result of evaporation with the acid. This solution was again decolorized by treatment with hydrogen peroxide as before. To the colorless solution, which showed only slight acidity, was added Lloyd's reagent to remove the nicotinic acid so that the remaining purification operations could be carried out by Dann and Handler's method of preparing the extract. The color development was made with aniline by the same method as with the first aliquot. This sample gave a nicotinic acid content of 7.88 mg per pound, a value in good agreement with the results of 7.44 mg per pound obtained by the Bina, Thomas, and Brown method.[1]

Furfuraldehyde: The successful oxidation with hydrogen peroxide

[1] It is essential that all of the hydrogen peroxide be removed from the solution prior to color development, to prevent oxidation of the reagent. The evaporation of the acid solution produces color that should be removed from the solution before color development. It was observed that the excess hydrogen peroxide used in the oxidation is not readily removed from the solution since sufficient amounts are carried over by the Lloyd's reagent to oxidize to the yellow oxide some of the lead nitrate used in the final purification step.

of the interfering chromogen produced by treatment with strong hydrochloric acid or strong caustic solution suggests aldehydes as the cause of this interference. It is well known that aldehydes are readily produced from cereal products by heating with hydrochloric acid and that furfuraldehyde and aniline react to give a colored complex.

Kodicek (1940), as well as others, calls attention to the possible interference from aldehydes, if present, in this reaction, but gives no specific data that would lead one to suspect that aldehydes are the source of this interference. In order to ascertain what effect furfuraldehyde, if present, would make on the determination, tests were made as follows:

A solution of furfuraldehyde was prepared and dilutions made so that varying amounts of this aldehyde could be tested by treatment with cyanogen bromide and the aromatic amines—aniline, metol, and *p*-aminoacetophenone—as are used in the determination of nicotinic acid.

Furfuraldehyde produces a strong coloration when treated with cyanogen bromide and aniline and is a potent chromogen if present in the extract where aniline is used. A solution containing 1 part in 15,000 gives rise to a large reading on the galvanometer and is read as nicotinic acid in the determinations using this reagent. The same is true with metol where a characteristic yellow color is produced that would give interference. Furfuraldehyde does not produce a color complex when treated with cyanogen bromide and *p*-aminoacetophenone, and hence offers no interference with this amine. The blank value and the solution value were identical with *p*-aminoacetophenone.

The fact that furfuraldehyde does not produce a color complex with cyanogen bromide and *p*-aminoacetophenone shows that this aldehyde is not identical with the chromogen responsible for the high results in the Melnick, Oser, and Siegel extract. That chromogen gave a color complex when treated with cyanogen bromide and *p*-aminoacetophenone, but it was not extractable with ethyl acetate.

Wheat germ: A sample of wheat germ was used for the next set of experiments. In these experiments the nicotinic acid content of wheat germ was determined by three different procedures, namely those of Bina, Thomas, and Brown, who employed takadiastase and a mild acid hydrolysis for the preparation of the extract and *p*-aminoacetophenone in the color development; Melnick, Oser, and Siegel, who employed concentrated hydrochloric acid for hydrolysis of the extract, and aniline in the color development; and Kringstad and Naess (1939), who employed a mild acid hydrolysis with dilute sulfuric acid for the preparation of the extract, and aniline in the color development. The following results were obtained:

Bina, Thomas, and Brown	24.13 mg per pound
Melnick, Oser, and Siegel	36.24 mg per pound
Kringstad and Naess	24.46 mg per pound

The results obtained by the procedures of Bina, Thomas, and Brown and Kringstad and Naess are practically identical but the results obtained by the procedure of Melnick, Oser, and Siegel are considerably higher and contain material other than nicotinic acid.

An aliquot of the extract prepared by the Kringstad and Naess method of hydrolysis was used for color development by the Melnick, Oser, and Siegel procedure of color development and a value of 24.06 mg per pound was obtained. This shows that the color complex responsible for the high value in the Melnick, Oser, and Siegel extract is not present in the extract prepared by the Kringstad and Naess procedure.

Sulfuric acid hydrolysis: Another aliquot of this extract (Kringstad and Naess) was subjected to hydrolysis by adding sulfuric acid to make a 15% solution and heating for two hours in a boiling water bath. Kringstad and Naess found this treatment would convert any nicotinic acid amide in the solution to nicotinic acid. The excess sulfuric acid was removed with barium hydroxide and the nicotinic acid determined as before the treatment. A value of 21.92 mg per pound was obtained. This result shows a small loss of nicotinic acid rather than the creation of an interfering compound in the reaction. A voluminous precipitate of barium sulfate is obtained in the neutralization of the sulfuric acid with barium hydroxide, and it is possible that the small difference in the results can thus be accounted for.

An aliquot of the solution as treated above with 15% sulfuric acid was evaporated to dryness, hydrolyzed with 10 ml of concentrated hydrochloric acid, and the nicotinic acid determined by the Melnick, Oser, and Siegel procedure. Only a slight increase in color development took place with this treatment; a value of 22.65 mg per pound was obtained on this solution compared to 21.92 mg per pound before the treatment.

Nicotinamide: In order to prove that a mild acid hydrolysis, similar to that employed in the procedure of Bina, Thomas, and Brown, is sufficient to convert nicotinamide to nicotinic acid, a sample of pure nicotinamide was analyzed. When 0.4 mg of this amide was subjected to the procedure of Bina, Thomas, and Brown, 0.42 mg of nicotinic acid was obtained. Since the molecular weights of nicotinic acid and nicotinamide are approximately the same, this result shows that the conversion of the nicotinamide to nicotinic acid was complete.

Alkaline hydrolysis: An extract as prepared by the Melnick, Oser,

and Siegel procedure was subjected to alkaline hydrolysis by first neutralizing the solution and then adding barium hydroxide to make a 10% solution. This solution was heated in a boiling water bath for 30 minutes and the barium removed with sulfuric acid. Considerable color developed in the solution, and it was evaporated to dryness and decolorized with nitric acid as previously described. The residue was dissolved in a known amount of water and aliquots were used for the determination of nicotinic acid by the color techniques of different methods. The results show that the previous value of 36.24 obtained by the Melnick, Oser, and Siegel procedure was reduced to 29.24 by this treatment.

The values of 27.80 and 26.27 obtained on this solution by the Kringstad and Naess and the Bina, Thomas, and Brown color techniques were somewhat higher than the values of 24.46 and 24.13 obtained on the extracts when prepared by their respective methods. This treatment removed a considerable portion of the interfering color complex developed in the Melnick, Oser, and Siegel procedure, but not all of it.

Nicotinic acid content of milk powder: Samples of skim-milk powder and whole-milk powder were used for determinations of nicotinic acid by different methods, and the results compared. The dry skim-milk powder gave 12.25, 20.30, and 12.50 mg per pound, respectively, by the Bina, Thomas, and Brown, by the Melnick, Oser, and Siegel, and by the Dann and Handler methods of analysis. The high value by the Melnick, Oser, and Siegel method is similar to that shown in the comparative results obtained on cereal products by their procedure in which interfering chromogens were shown to be the cause of the high results. This is confirmed by the fact that their extract showed a lower value, 14.5 mg per pound, when the color technique of the Bina, Thomas, and Brown procedure was applied.

Whole-milk powder gave correspondingly lower values than the skim-milk powder, because of the high fat content. A value of 9 mg per pound was obtained on this sample of whole-milk powder.

In the analysis of milk products turbidity is encountered unless special precautions are taken to remove the precipitate that continues to deposit after the neutralization of the extract. We find that this interference is removed if the neutralized extract is cooled in an ice bath for a period of 30 minutes and then centrifuged at high speed just prior to color development.

Discussion and Summary of Results

From the results we have obtained by different procedures for the determination of nicotinic acid in cereal products, yeast, and milk,

we have found that the differences obtained are due mainly to the hydrolysis used in the preparation of the extracts. These results show that strong acid or alkaline hydrolysis is not necessary for the complete extraction of the nicotinic acid in cereal products, but on the contrary is detrimental in that the chromogens and other coloring materials are produced that interfere with the nicotinic acid determination. The nicotinic acid content of cereal products is completely extracted by autoclaving a water suspension and subsequently treating it with taka-diastase. Any amide present is completely converted to the acid by a mild acid hydrolysis without the production of interfering chromogens and with a minimum of color in the extract.

Decolorizing techniques employed by the different procedures for treatment of the extracts proved of little advantage to the method. With the exception of hydrogen peroxide none of the decolorizing treatments did more than remove color that was compensated for by the blank. Some of the procedures were more effective than others for producing water-clear extracts. Decolorizing with charcoal was incomplete and ineffective. The decolorizing technique of Dann and Handler with Lloyd's reagent and lead nitrate produces colorless extracts with very small blank values; however, this treatment does not free the extract from interfering chromogens. Hydrogen peroxide is an efficient decolorizing reagent that will remove interfering chromogens as well as decolorize the solution. Its use for this purpose is not recommended, however, because of difficulty in removing the excess peroxide. If extracts of cereal products are properly prepared, satisfactory solutions will be obtained without the use of decolorizing reagents.

Aniline, metol, and *p*-aminoacetophenone were used in these experiments and gave the same nicotinic values for yeast only, where no interfering substances were produced by any of the procedures tried. Aniline and metol were not found suitable for use in the determination of nicotinic acid in cereal products and milk where interfering substances were produced in the hydrolysis with strong acid and alkali. The use of *p*-aminoacetophenone and ethyl acetate gave consistent results on all of these products for the nicotinic acid content, and the values obtained are in accord with the low anti-pellagric properties found by animal experimentation on these products.

These experiments show the presence of an interfering chromogen in extracts of cereal products prepared by strong acid or alkaline hydrolysis. This chromogen is colorless in solution prior to treatment with cyanogen bromide and the aromatic amine. With metol and cyanogen bromide this chromogen produces a dark brown color that masks the lemon yellow color produced by nicotinic acid and this

amine. This substance produces a color with aniline and cyanogen bromide similar to the color produced by nicotinic acid and is read on the fluorophotometer, thus giving rise to misleading results for nicotinic acid where aniline is used. The color complex produced by this chromogen with *p*-aminoacetophenone and cyanogen bromide is not soluble in ethyl acetate and hence is not extracted by this solvent to interfere in the color reading of the nicotinic acid. Lloyd's reagent precipitates this chromogen completely from the extract along with the nicotinic acid and it is brought back into the solution by the caustic. The subsequent decolorizing action of lead nitrate serves only to remove color normally eliminated by the blank and hence reduces high blank values but does not eliminate this chromogen as an interfering substance. This chromogen is oxidized by hydrogen peroxide.

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THE UTILITY OF MICRO METHODS OF TEST-WEIGHT DETERMINATION WITH HARD RED SPRING WHEAT [1]

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Micro methods of analysis are becoming increasingly important to the scientist. Valuable time and material may be conserved by these techniques, and in the field of cereal technology the introduction of micro methods of experimental milling and baking has enabled the technologist to obtain trustworthy data from wheat samples too small to be tested by the older procedures. These methods are especially valuable in nutrition investigations, as well as in preharvest quality surveys, where only small quantities of wheat are available, as in wheat breeding work. The same situation exists when wheat is grown in nutrient cultures where the cost of space and chemicals limits the amount of wheat that can be produced.

In working with these small samples of wheat the authors have found it necessary to devise some method whereby the test weight per bushel could be ascertained without the use of the standard method described in the *Handbook of Official Grain Standards* published by the Agricultural Marketing Service, U. S. Department of Agriculture. This standard method is based upon the use of a quart measure and required more than a quart of wheat before the test may be employed. By definition of the U. S. Department of Agriculture the test weight of a sample of grain is the weight of the volume of grain required to fill level full a Winchester bushel of 2,150.42 cubic inches capacity. This value is very important in wheat grading, as definite limits in test weight exist among the different grades in sound and unmixed wheats. As grade controls the price, test weight is highly significant to the wheat grower and is therefore of interest to the agronomist and cereal technologist when determining varietal effects upon wheat quality. It is also a factor of importance to the flour manufacturer since it is positively correlated with flour yield.

Micro test-weight technique was used by the Agricultural Marketing Service in a preharvest wheat survey in the hard red spring wheat region in the 1939 season and in the hard red winter wheat region in both 1939 and 1940. In this method a small coin envelope was used to hold the wheat. This envelope was held two inches above the top of a cut-down graduated cylinder when filling. The graduate was tapped lightly three times with a stroker before striking off the wheat

[1] Published with the approval of the Director of the Station.

level with the top of the graduate, which was 16 ml in capacity. The wheat was then weighed and the factor 5.0 used to calculate the weight per bushel from the weight in grams of the sample.

Aamodt and Torrie (1934) studied the relationships between the weight (g × 20) of a 4-ml wheat sample and the weight in pounds per bushel as determined by the usual method, except that an Imperial pint measure was employed instead of the official quart kettle. A cut-down 25-ml graduated cylinder was employed to measure the wheat. The grain was poured into the measure from a coin envelope, pressed down lightly with the thumb, and leveled off with a scalpel drawn across the edges of the measure. It was found that this micro method yielded a close approximation to the test weight per bushel. Correlation coefficients of +.947 for 184 samples of spring wheat and +.834 for 59 samples of winter wheat were obtained between the values found by the two methods. The authors pointed out the utility of the micro method in determining test weight in experiments dealing with small samples harvested from pot cultures in plant nutritional and disease studies and in plant breeding investigations. Weight per 1,000 kernels lacked reliability when different-sized wheat kernels were involved, and the determination was too tedious and expensive when a large number of samples were to be tested.

Harris and Sibbitt (1941) described a procedure for determining test weight in which 4-ml and 16-ml measures were used. These measurements were compared with the test weight obtained by the standard official method using the quart kettle. Lower values were noticeable in the results obtained when the weight in grams of 4 ml of grain was multiplied by the conversion factor of 20 used by Aamodt and Torrie, despite the fact that the bushel in use in Canada contains 36,369 ml, while the Winchester bushel employed in the United States contains only 35,239 ml.

No doubt the discrepancy found between the 4 ml and the standard-method results by Harris and Sibbitt and those reported by Aamodt and Torrie is due to differences in technique in making the micro determinations. The data appeared to indicate the possibility of successfully using micro methods in accurately determining the test weight per bushel of hard red spring wheats, and pointed to the advisability of further investigations of the methods employed with the end of devising by statistical methods a suitable factor or formula to employ in calculating test weights from micro methods. Because of the small number of observations (triplicate tests on ten samples) reported in the preliminary study, it would also be necessary to obtain more comprehensive data before suitable formulas could be worked out.

Materials

The first experiment undertaken was set up to determine the relative degrees of decision in predicting test weight per bushel from the following measurements carried out on very small samples of wheat: (1) weight in grams of 16-ml and 4-ml portions of the wheat, (2) weight of 16-ml and 4-ml portions of ground wheat meal, and (3) weight of 500 kernels.

It was also thought desirable to find out whether there is an "operator" effect—in other words, whether the method in the hands of one worker would yield significantly different results from those obtained by another person. Twenty-two samples of wheat from the field-plot variety trials were included in the series used in this part of the study. These wheats consisted of a number of hard red spring varieties. Thirty samples were also included from the Crop Weather Studies which are carried on at this Station in cooperation with the Agricultural Marketing Service. The latter group consisted of three varieties only—Thatcher, Ceres, and Premier. All tests carried out on this series of wheat were done in triplicate.

A second series, consisting of regular field-plot variety and other miscellaneous samples, were used to obtain a further check upon the relationship between the standard-quart and 16-ml techniques. This set numbered 134 samples, and the 16-ml determinations were made in quintuplicate, while the quart readings were taken but once as it was felt that the greater variability would exist in the micro method.

A third series of wheat was selected consisting of 140 samples from a wheat-breeding nursery. These samples were small in size and it was therefore impossible to carry out determinations by the standard method, which requires in excess of one quart of wheat. This series contained a great diversity of hard red spring types and was extremely variable in nature. Micro test-weight measurements were made with the 16-ml and 4-ml measures, in order to obtain further data on the correlation between the two procedures.

In the work at this Station the 16-ml method would be by far the most widely used of the micro methods. The 4-ml technique would only be resorted to in a limited number of cases where sufficient material was not available for the 16-ml method. This situation would arise at this Station only in plant nutrition studies.

Equipment Used for Micro Methods

In order to obtain comparable results with any method of micro weight determinations it was imperative to standardize strictly the equipment and procedure used. Glass graduated cylinders of 100- and 25-ml capacity were cut down to suitable size and the edges care-

fully ground smooth to contain accurately the desired quantity of wheat. The wheat was placed in a funnel made of heavy tin sufficiently large to hold an excess of wheat over that required to fill either measure. The funnel was fitted with a stand to hold it steady at a constant height of one inch above the center of the measuring cylinder. The lower opening of the funnel was ½ inch in diameter and was equipped with a shutter to control the flow of the wheat. Preliminary tests with the 16-ml measure were conducted on two different types of stroker to determine which would be best suited for the micro method. One stroker was flat, ⅝ inch in width and 7 inches long, with rounded edges. It closely resembled the one recommended in the official standard methods. The other stroker was round, with a diameter of ⅜ inch and a length of 7 inches. Tests made with a small series of samples showed better agreement when the round stroker was used, and it was accordingly adopted for use in these investigations. A grain-testing balance with a sensitivity of 10 mg was used for weighing the measured wheat. The equipment used in conducting the micro tests is shown in Figure 1.

Fig. 1. Equipment used in determining micro test weights. Funnel fitted with shutter and the 16- and 4-ml measures are shown in center, with the quantities of wheat required for each test. Weighing balance at right, stroker in front of measures.

Technique of Determination

The method used in the determinations was essentially the same for both the micro methods and has been described by Harris and Sibbitt (1941) in some detail. The wheat to be tested is placed in the funnel and the measure carefully positioned under its delivery end. The funnel shutter is then quickly opened and the entire quantity of grain allowed to run into the measure, filling it to overflowing. The stroker is then placed lightly on the edge of the measure, care being

taken not to jar or disturb the cylinder and its contents. The wheat is next leveled off with a zigzag motion of the stroker. The quantity of wheat remaining in the cylinder is then weighed.

The weight per 500 kernels was also determined in the first series, as this value has been considered to be related to kernel plumpness and test weight. It has been extensively used by agronomists and others interested in the quality of wheat.

As some difference had been experienced in connection with the packing of the kernels in the measure, especially when the 4-ml method was used, it was thought desirable to obtain information respecting the possible use of wheat meal as material for predicting test weight by micro methods. Air spaces between the kernels and the container would be eliminated, and a strictly level surface should be obtained following striking off. These factors would be more operative when the 4-ml method was employed. To settle this question a sufficient quantity of each sample in the first series was ground on a small Wiley mill to pass through a 20-mesh sieve, and micro test weights made on the resultant meal. As already pointed out a further set of tests was made on this series by two operators to determine whether there would be a significant difference in the two sets of data obtained occasioned by small variations in the procedure introduced by personal factors. The 16-ml micro method only was included in this study as this is the micro technique which is most useful in test-weight determinations in actual practice.

Discussion of Results

A scatter diagram of the 52 average results for the standard and 16-ml micro method is shown in Figure 2. It is clearly evident that a high positive correlation exists between the values yielded by the two methods. Similar diagrams are shown for the data from the standard and 4-ml micro method, as well as for the standard with the ground wheat meal by the 16-ml method in Figures 3 and 4, respectively. The relationship between the standard test weight and weight of 500 kernels is similarly represented in Figure 5. The regression line for predicting test weight (standard) from the weight in grams of material obtained by the various alternative micro methods is shown in these diagrams. The degree of correlation between the test weight and the other values shown decreases in the order named, with little relationship of importance apparent in Figure 5. The correlation coefficients obtained with corresponding fiducial limits, prediction equations, and standard errors of estimate are found in Table I.

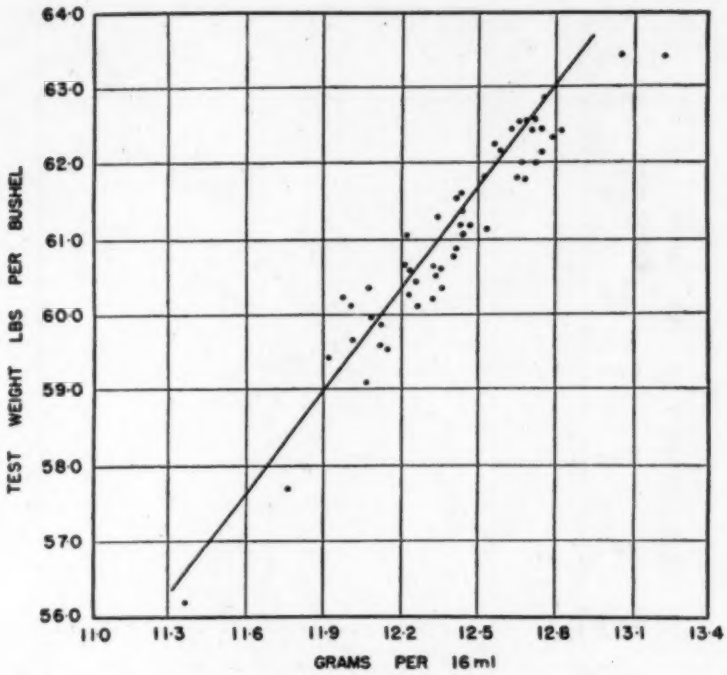

Fig. 2. Dot diagram of weight in grams of 16 ml of wheat and test weight per bushel. $N = 52$. $r = +.9539$.

TABLE I

CORRELATION COEFFICIENTS, WITH FIDUCIAL LIMITS, PREDICTION EQUATIONS AND ERROR OF ESTIMATES COMPUTED FROM DATA OF SERIES 1

Variables correlated		Correlation coefficients r_{xy}	Fiducial limits		Prediction equation	Standard error of estimate
X	Y		Lower	Upper		
Test weight, standard method, lbs	Micro 16-ml method, g	+.9631	+.9407	+.9875	$4.56y + 4.7$	0.365
"	Micro 4-ml method, g	+.8824	+.7616	+.9343	$21.05y - 0.05$	0.638
"	Micro 16-ml method, g (ground wheat meal)	+.6951	+.5169	+.8155	$3.38y + 29.9$	0.977
"	Micro 4-ml method, g (ground wheat meal)	+.7028	+.5200	+.8210	$13.24y + 31.0$	0.966
"	Weight 500 kernels, g	+.5592	+.4186	+.7246	$0.38y + 56.1$	1.126

Fiducial limits represent the upper and lower limits according to a given probability level between which the true value of a statistic lies. In the present instance this probability level is the 5% point. These values are calculated from the distribution of z, rather than from that of the correlation coefficient r, and they are accordingly not subject to any uncertainty due to lack of normal distribution, as is the case for

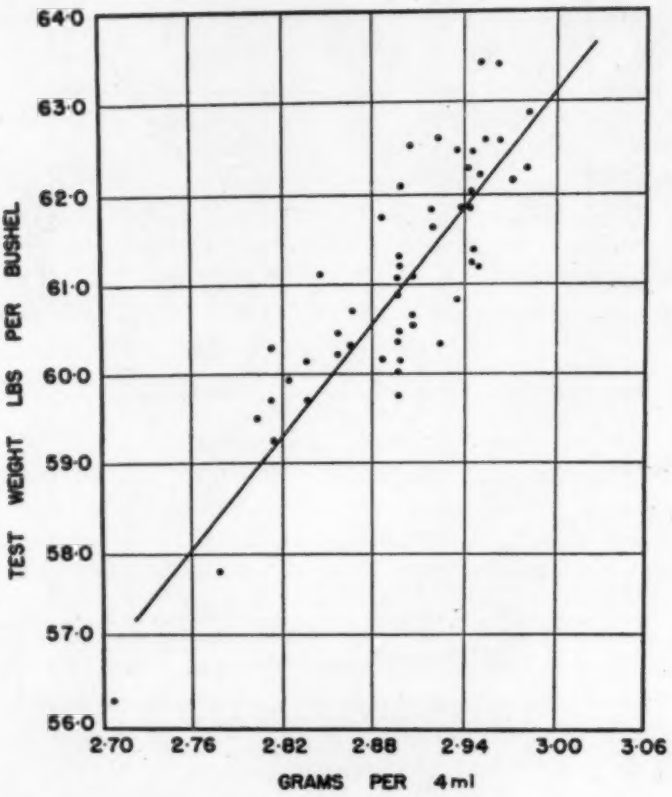

Fig. 3. Dot diagram of weight in grams of 4 ml of wheat and test weight per bushel. $N = 52$. $r = +.8332$.

certain values of the correlation coefficient, especially in the instance of small samples.

Table II shows the analyses of variance for the data obtained by

TABLE II

ANALYSIS OF VARIANCE OF OPERATOR EFFECT IN MICRO TEST WEIGHT DETERMINATIONS

Source of variance	Sum of squares	Degrees of freedom	Variance	F	5% point	1% point
Between operators	0.151	1	0.151	37.8	3.89	6.76
Between samples	15.298	51	0.300	75.0	—	—
Interaction (operators × samples)	10.532	51	0.203	50.8	—	—
Sampling error	0.806	208	0.004	—	—	—
Total	26.787	311	—	—	—	—

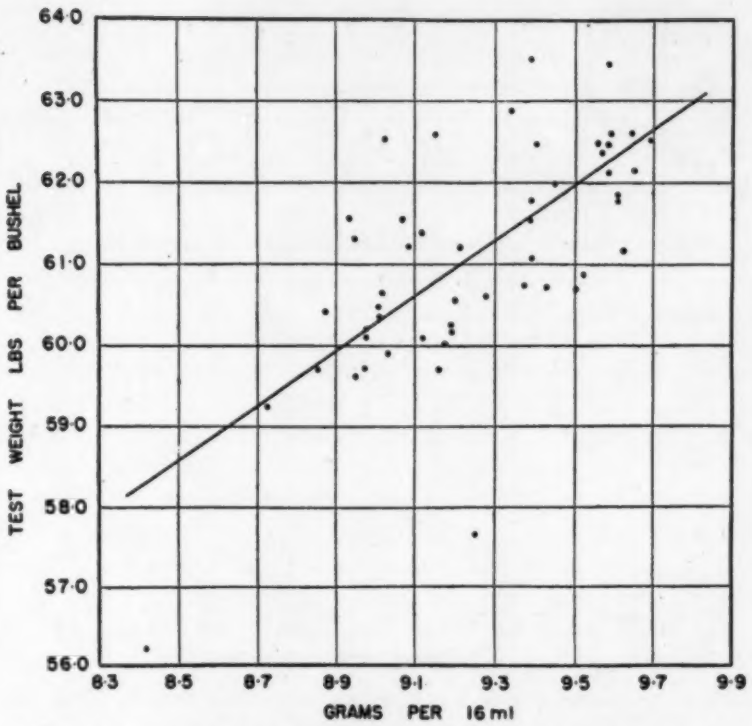

Fig. 4. Dot diagram of weight in grams of 16 ml of ground whole wheat meal and test weight per bushel. $N = 52$. $r = +.6933$.

the two different operators. As the F value computed is very much higher than the value at the 5% and 1% points the difference is clearly significant. This difference in results obtained by different operators is being thoroughly investigated at the present time, by four workers.

The second series of tests resulted in a correlation coefficient of +.9286 between the standard-quart and the 16-ml micro methods, as shown in Table III. This statistic is in good agreement with the co-

TABLE III

CORRELATION COEFFICIENTS, WITH FIDUCIAL LIMITS, COMPUTED FROM THE DATA OF SERIES 2 ($N = 134$) AND SERIES 3 ($N = 140$)

Variables correlated		Correlation coefficients r_{xy}	Fiducial limits		Standard error of estimate
X	Y		Lower	Upper	
Test weight, standard method, lbs...	Micro 16-ml method, g	+.9286[1]	+.9011	+.9486	0.768
Micro 16-ml method, g.....	Micro 4-ml method, g	+.9030	+.8841	+.9195	—

[1] Prediction equation = $4.48y + 5.5$.

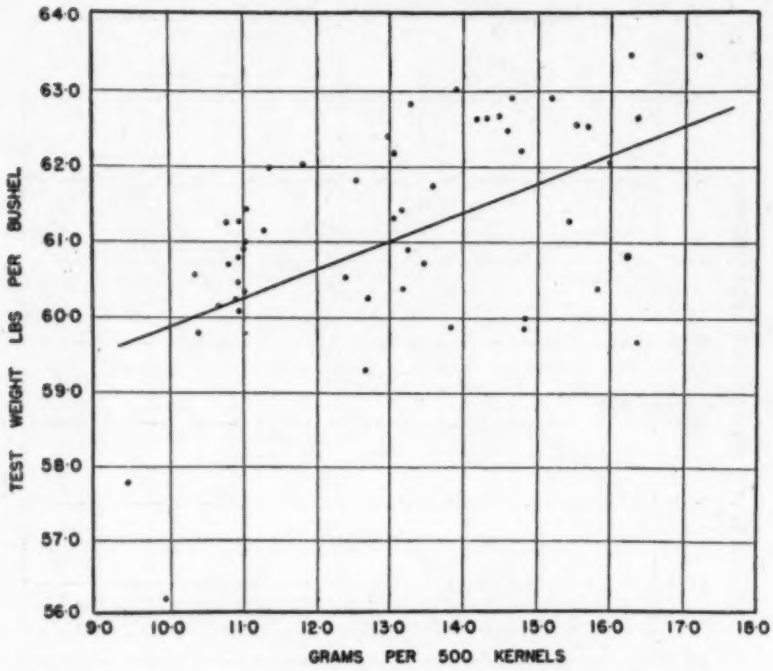

Fig. 5. Dot diagram of weight in grams of 500 kernels of wheat and test weight per bushel. $N = 52$. $r = +.5547$.

efficient found between the corresponding variables in the first series, and emphasizes the reliability of the test for the purpose of predicting test weight per bushel from small samples of wheat. The prediction equation, based on the two sets of data, yields results differing by not more than 0.2 lb in predicted weight per bushel within the usual range of test weights.

Table III contains also the statistical data from the third series, in which a comparison was made between the two micro methods. The results show that the weights obtained by the two methods are highly correlated, but as no attempt would probably ever be made to determine the weight in grams for the 16-ml method from the results of the 4-ml, no prediction equation is included in the data obtained from this series.

From the results described in this paper it is apparently possible to predict the weight per bushel as found by the usual official procedure from the weight in grams of 16 ml of the same wheat. It is also possible to predict, with somewhat less accuracy, the bushel weight from the weight in grams of 4 ml of the wheat. Ground wheat meal is not satisfactory as material for test-weight determinations, and the weight of 500 (or 1,000) kernels is quite unsatisfactory for prediction purposes.

Summary and Conclusions

Procedures have been described for the estimation of the test weight per bushel of hard red spring wheat samples too small to be tested by the official standard method. These methods include the weight in grams of: (1) 16 ml of wheat, (2) 4 ml of wheat, (3) 16 ml of finely ground wheat meal, (4) 4 ml of finely ground wheat meal, and (5) 500 kernels.

The 16-ml method gave the most satisfactory results, in respect to the degree of correlation with the standard method. The 4-ml method was the next best. Both these methods have distinct utility for the determination of test weight from small samples of wheat. The methods employing ground wheat meal were not highly correlated with test weight and would accordingly not be satisfactory for prediction purposes. The weight of 500 (or 1,000) kernels would be still less reliable for finding bushel weight.

Comparative determinations made by two operators on the same series of wheats with the 16-ml method showed significant differences between operators. This point is being further investigated. Suitable charts or tables can be constructed from which the standard test weight per bushel may be read off directly from micro results in grams. The findings confirm the usefulness of micro test-weight determinations as used at this Station.

Acknowledgments

The authors wish to acknowledge the assistance and advice of Clifford Maloney in the evaluation of the data. Thanks are also due to Works Projects Administration, Research and Records Division, through the operation of Seed Testing Project, O.P. 165-1-73-144, during this investigation, as well as to NYA funds for technical assistance. Mention should further be made of the Agricultural Marketing Service, U. S. Department of Agriculture, for their encouragement of the project, and to Dr. E. A. Helgeson, Head, Department of Botany, North Dakota State College, for his keen interest in the work.

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A MICRO METHOD FOR DETERMINING TEST WEIGHT[1]

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Micro methods for the determination of test weight or pounds per bushel are needed in studies on wheat properties whenever the amount of grain available is insufficient for standard methods. Plant breeders need such methods because the amounts in available samples are small. Preharvest survey workers must use such methods because of limitations in procuring the larger samples. In estimating the relative ratios of kernel sizes in milling wheat in order to determine the relation of these sizes to probable flour yield and test weight, micro test weight methods will save much labor in making the separations.

There is but little information available on micro methods for determination of test weight. Aamodt and Torrie (1934) devised a method requiring 4-ml samples. The weight in grams of a 4-ml sample multiplied by 20 was found to give a close approximation to the test weight in pounds per bushel as determined by the usual apparatus. These investigators state that "The correlation coefficient calculated from the weight per bushel of 184 samples of spring wheat by these two methods was $+ .947 \pm .005$ and for 59 samples of winter wheat $+ .834 \pm .027$."

A study of micro test weight determination prepared by D. E. McCarty and J. W. Kirkbride is contained in a private communication from Dr. A. J. King, Agricultural Statistician of the Agricultural Marketing Service. Two brass cylinders of 16.08 ml and 32.5 ml capacity, respectively, were used. The test weights obtained by the micro method were somewhat smaller than the official method but they were sufficiently in agreement to warrant their use for research and some other purposes where the amount of material is insufficient to be used for the official method, Dr. King reported.

The need for a micro test weight method was revealed in the study of the effects of moisture on the physical properties of wheat (Swanson, 1941), because much labor could be saved by using small samples. A method was desired which would require only about 100 g of wheat. Three cups were made by cutting off the upper portions of cylindrical glass sample bottles and grinding a smooth edge. The cups were designated A, B, and C. The inside diameters of A and B were 5.4 cm, but A was 0.6 cm taller than B. The inside diameter of C was 4.4

[1] Contribution No. 80, Department of Milling Industry.

cm and the height was 1.9 cm taller than B. The volume capacities of the cups were: A, 106.5 ml; B, 91.5 ml; and C, 89.8 ml. Cup A held about 80 g, cup B about 69 g, and cup C about 68 g of wheat, depending on the test weight. It was planned that cup A should approximate the same ratio of depth to diameter as found in the official brass quart measure, and then have B with a lower height and C taller but with less diameter. This would give variations in capacities as well as ratios of diameters to depths to find which size agreed most nearly with the results of the official test weight method.

Formula for Calculation of Test Weight

The ratio between 35,238 ml in a bushel and 453.6 g in a pound is 77.69. Therefore, if a cup of the correct height and diameter had a volume of 77.69 ml, the weight in grams of wheat in this cup level full would be equal to the test weight in pounds per bushel. Since the cups used in this study had greater capacities, 77.69 divided by the capacities of the cups would give the factors by which to multiply the weight of wheat to obtain the test weights in pounds per bushel. These factors were: cup A, 0.73; cup B, 0.85; and cup C, 0.865. A table was calculated to permit rapid and direct readings.

Method of Procedure

A funnel through which the wheat was poured into the cup was obtained by cutting a glass funnel of 9.0-cm diameter a little above the juncture of the cone and stem and grinding to give an opening 1.4 cm in diameter. It is important that this opening be large enough to allow the wheat to flow freely but not too rapidly. The funnel is held in an ordinary ring stand and adjusted so that its opening is approximately two inches above the top of the cup. The cup is placed in a shallow pan to catch the overflow grain. The time for filling the cup should be such that the wheat kernels have somewhat the same freedom to flow and settle as in the official cup. In making a determination, the finger is held against the opening of the funnel so as to delay the flow of wheat until the funnel is full. The stroking or leveling of the wheat after the cup is filled to running over is made by a small ruler.

Statistical Analysis

With the method as outlined, the test weights of all the wheat samples used in the investigation mentioned (Swanson, 1941) were determined in duplicate for each of the cups A, B, and C. Since these wheat samples varied considerably in the official test weights they afforded a good basis to test the accuracy of the method.

Both official test weights and those obtained by cups A, B, and C were subjected to statistical analysis.[2] From this analysis of the test weights taken on the 95 samples by the official method and by the micro method in duplicate, using in turn cups A, B, and C, the following conclusions were reached:

1. The test weights obtained with cup A were significantly lower than the official.

2. The test weights obtained with cup B were slightly lower than the official, but the differences were not significant.

3. The test weights obtained with cup C were slightly higher than the official, but the differences were not significant.

4. The grand averages in pounds of the test weights of all the samples were: official 57.42; with cup B, 57.10; with cup A, 56.86; with cup C, 57.65.

5. The averages and standard errors were as follows: official, 57.42 ± 0.17; with cup A, 56.83 ± 0.17; with cup B, 57.09 ± 0.177; with cup C, 57.63 ± 0.200.

The reasons for these variations were not investigated.

The study shows that a micro test weight method suitably designed is sufficiently accurate when only small samples of grain are available.

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[2] The writer is indebted to Mr. John A. Johnson, Jr., and Mr. William W. O'Donnell, Assistant and Research Assistant in the Department of Milling Industry, for making the statistical study.

THE EFFECT OF TEMPERATURE CHANGE DURING MALTING ON FOUR BARLEY VARIETIES [1]

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(Read at Annual Meeting, May 1941)

In an earlier experiment with a small malting chamber, four barley varieties were malted at constant temperatures of 12°, 16°, 20°, and 24°C, respectively, for periods of two, four, six, and eight days, with two moistures, one relatively low and the other rather high. The results of this series were reported by Shands *et al.* (1941), who suggested that conditions which were changed systematically or periodically might more expressly influence malt quality. It also was realized that commercial malting is generally done under conditions where temperature is not held constant but changed at certain intervals. In view of these observations a series of 72 samples was malted in the small chamber, and temperature was changed at regularly scheduled time intervals. Under this set of conditions it was possible to observe the effects of several combinations using different time intervals at different temperatures and to make comparisons with malts produced under constant temperatures.

Plan of Experiment

The plan of the experiment here reported was to malt four barley varieties six days, using an intermediate moisture (45%) and the temperatures 12°, 16°, and 20°C singly and in varied sequence. Fifteen malting methods listed in Table I may be summarized as follows: three of the methods involved constant temperatures and 12 involved operations in which the above temperatures were shifted at time intervals of two or four days. For example, if a sample was malted at 12°C for two days, the temperature might be changed to either 16° or 20°C for the remaining four days. Or a sample might be malted for four days at 20°C and then changed to 16° or 12°C for the remaining two days. Duplicate maltings were made of the constant-temperature series, while single malts were produced in the temperature-change series.

[1] Based on cooperative investigations between the Wisconsin Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The Federal WPA has contributed to the research program through a grant under the University of Wisconsin WPA Natural Science Project. The United States Maltsters Association has cooperated through an industrial fellowship grant to the University of Wisconsin.

TABLE I

PHYSICAL AND CHEMICAL ANALYSES OF MALT PRODUCED IN SMALL CHAMBER IN SIX DAYS AT CONSTANT TEMPERATURES COMPARED WITH MALTS PRODUCED IN SIX DAYS WITH TIME AND TEMPERATURE CHANGED

Malting time and temperature	Method no.	Steep and respiration loss	Recovery	Growth index	Kernel weight malt	Extract, dry basis	Color	Diastatic power	Time of conversion	Malt nitrogen	Ratio of wort nitrogen to malt N	Ratio of formol nitrogen to malt N	Wort acidity
		%	%	*no.*	*mg*	%	*Lov 52*	°*L*	*min*	%	%	%	*pH*
A. ODERBRUCKER													
6 days at 12° C (constant)[1]	1	4.1	93.1	81.3	28.6	75.5	1.2	170	7.3	1.99	36.2	14.2	6.1
6 days at 16° C (constant)[1]	2	5.0	90.3	85.0	28.5	74.6	1.4	177	<5	1.98	34.4	13.6	6.0
6 days at 20° C (constant)[1]	3	6.6	88.6	86.9	27.7	73.8	1.4	172	6.8	2.00	35.2	12.0	5.9
4 days at 12°, 2 days at 16° C	4	3.9	92.4	78.5	28.9	74.7	1.3	174	5–7	1.99	34.3	13.4	6.0
2 days at 12°, 4 days at 16° C	5	4.5	91.4	78.0	28.4	74.9	1.3	166	5–7	1.97	33.9	13.9	6.1
4 days at 12°, 2 days at 20° C	6	4.9	91.3	80.3	28.5	75.0	1.5	172	5–7	1.97	34.0	12.6	5.9
2 days at 12°, 4 days at 20° C	7	6.2	89.0	86.8	28.0	73.6	1.6	175	<5	1.96	32.9	12.1	5.5
4 days at 16°, 2 days at 12° C	8	4.6	92.2	86.0	28.8	75.3	1.5	189	<5	1.96	37.1	15.2	6.0
2 days at 16°, 4 days at 12° C	9	4.3	92.5	78.5	28.9	75.5	1.5	186	<5	1.98	37.1	15.0	5.7
4 days at 16°, 2 days at 20° C	10	5.8	89.1	82.5	28.4	74.5	1.3	180	5–7	2.00	33.6	11.9	6.0
2 days at 16°, 4 days at 20° C	11	5.9	89.0	78.3	28.1	74.0	1.4	174	5–7	1.99	32.0	12.2	5.8
4 days at 20°, 2 days at 12° C	12	6.0	89.6	90.3	28.5	74.8	1.4	170	7–10	1.97	34.8	13.5	6.1
2 days at 20°, 4 days at 12° C	13	5.5	91.1	99.0	28.3	75.3	1.4	180	7	1.91	40.0	16.1	6.2
4 days at 20°, 2 days at 16° C	14	6.4	89.8	84.5	27.8	74.2	1.6	177	<5	2.00	32.0	12.3	6.0
2 days at 20°, 4 days at 16° C	15	5.1	91.4	89.5	28.2	74.4	1.5	171	<5	1.99	36.1	13.9	6.1
B. WISCONSIN BARBLESS													
6 days at 12° C (constant)[1]	1	3.6	93.8	86.2	29.3	73.6	1.3	114	10–15	1.96	29.3	11.2	6.1
6 days at 16° C (constant)[1]	2	4.7	91.4	85.0	28.4	73.3	1.3	115	10	1.93	29.3	11.3	6.0
6 days at 20° C (constant)[1]	3	5.8	89.3	84.2	27.9	72.6	1.4	124	10–15	1.95	31.8	9.8	5.9
4 days at 12°, 2 days at 16° C	4	3.1	93.7	72.3	28.6	73.0	1.1	109	10–15	1.92	28.0	10.9	6.0
2 days at 12°, 4 days at 16° C	5	3.7	92.6	72.0	28.6	73.2	1.3	113	10–15	1.93	26.2	11.3	6.2
4 days at 12°, 2 days at 20° C	6	4.3	92.1	78.5	28.8	73.2	1.1	120	10–15	1.94	28.0	10.2	6.0
2 days at 12°, 4 days at 20° C	7	5.5	90.0	85.0	27.8	72.4	1.6	120	10	1.98	25.8	9.1	5.6
4 days at 16°, 2 days at 12° C	8	4.1	93.0	84.5	28.7	73.4	1.5	127	10	1.93	31.4	12.4	5.8
2 days at 16°, 4 days at 12° C	9	3.6	93.3	78.8	29.1	73.5	1.4	123	7–10	2.00	29.6	11.7	5.7
4 days at 16°, 2 days at 20° C	10	5.0	90.7	79.8	28.1	73.3	1.1	121	10–15	1.92	28.1	10.5	6.0
2 days at 16°, 4 days at 20° C	11	4.9	90.7	74.3	28.9	72.6	1.3	114	10–15	1.90	26.6	10.2	5.8
4 days at 20°, 2 days at 12° C	12	4.8	90.9	83.3	28.3	73.4	1.1	116	15	1.92	28.4	10.9	6.1
2 days at 20°, 4 days at 12° C	13	5.1	91.7	96.3	28.5	74.1	1.1	124	10–15	2.03	31.3	12.1	6.2
4 days at 20°, 2 days at 16° C	14	5.9	90.4	85.5	28.0	73.2	1.4	123	10	1.96	27.2	10.1	6.0
2 days at 20°, 4 days at 16° C	15	5.0	91.1	93.0	28.7	73.1	1.4	121	7	1.90	34.5	11.9	6.2

[1] Average of duplicate maltings.

[1] Average of duplicate maltings.

TABLE I (*Continued*)

Malting time and temperature	Method no.	Steep and respiration loss	Recovery	Growth index	Kernel weight malt	Extract, dry basis	Color	Diastatic power	Time of conversion	Malt nitrogen	Ratio of wort nitrogen to malt N	Ratio of formol nitrogen to malt N	Wort acidity
		%	%	*no.*	*mg*	%	*Lov 52*	°*L*	*min*	%	%	%	*pH*
C. PEATLAND													
6 days at 12° C (constant)[1]	1	4.1	92.9	81.7	21.8	74.4	1.4	159	7–10	2.09	36.2	14.0	6.1
6 days at 16° C (constant)[1]	2	5.4	90.5	81.3	20.8	74.1	1.6	172	7	2.08	38.0	14.9	6.1
6 days at 20° C (constant)[1]	3	7.2	87.8	86.1	20.6	72.8	1.8	156	6.3	2.14	36.2	12.8	5.9
4 days at 12°, 2 days at 16° C	4	3.8	92.7	74.5	21.3	74.3	1.5	153	5–7	2.06	37.1	14.6	5.9
2 days at 12°, 4 days at 16° C	5	4.5	91.2	72.8	21.5	74.0	1.6	156	7–10	2.06	37.9	15.1	6.2
4 days at 12°, 2 days at 20° C	6	5.5	90.1	79.8	21.5	73.9	1.5	162	7–10	2.08	36.3	13.8	6.0
2 days at 12°, 4 days at 20° C	7	6.7	88.0	90.0	21.0	72.7	1.9	157	<5	2.12	34.8	13.0	5.8
4 days at 16°, 2 days at 12° C	8	4.7	92.3	85.8	21.8	74.3	1.6	174	5	2.09	38.2	15.0	5.8
2 days at 16°, 4 days at 12° C	9	4.4	92.3	82.0	21.0	74.8	1.5	177	<5	2.09	39.8	16.0	5.8
4 days at 16°, 2 days at 20° C	10	6.2	88.9	80.0	20.9	73.5	1.4	158	7	2.10	34.6	13.2	6.0
2 days at 16°, 4 days at 20° C	11	6.5	88.5	74.8	20.4	73.5	1.8	150	7–10	2.14	33.4	13.0	5.8
4 days at 20°, 2 days at 12° C	12	6.2	89.2	89.5	20.6	73.8	1.4	145	10	2.11	36.0	14.1	6.1
2 days at 20°, 4 days at 12° C	13	6.2	90.4	97.3	21.3	74.3	1.5	172	10	2.17	37.4	15.2	6.2
4 days at 20°, 2 days at 16° C	14	7.1	88.8	84.3	20.8	73.5	1.9	155	5	2.10	35.6	13.6	6.1
2 days at 20°, 4 days at 16° C	15	5.8	90.3	87.3	21.1	73.5	1.9	153	5	2.18	36.7	14.0	6.2
D. CHEVRON													
6 days at 12° C (constant)[1]	1	4.1	93.1	79.4	22.3	74.0	1.4	208	5.5	2.27	36.9	14.2	6.1
6 days at 16° C (constant)[1]	2	5.6	90.0	79.7	20.8	73.4	1.6	224	<5	2.27	35.9	13.9	6.0
6 days at 20° C (constant)[1]	3	7.3	87.3	82.8	20.7	72.3	1.7	206	6.3	2.31	35.7	12.0	5.8
4 days at 12°, 2 days at 16° C	4	3.7	92.5	74.0	22.0	74.5	1.5	201	5–7	2.24	36.2	14.1	5.8
2 days at 12°, 4 days at 16° C	5	4.6	90.4	71.5	21.3	73.6	1.5	215	5	2.26	35.2	14.2	6.1
4 days at 12°, 2 days at 20° C	6	5.4	90.3	78.8	21.6	73.6	1.4	207	5–7	2.25	34.7	12.8	6.0
2 days at 12°, 4 days at 20° C	7	6.7	88.1	87.5	21.1	71.9	1.6	201	<5	2.27	34.0	11.8	5.7
4 days at 16°, 2 days at 12° C	8	4.8	91.7	80.5	21.7	74.0	1.8	239	<5	2.29	36.3	14.1	5.8
2 days at 16°, 4 days at 12° C	9	4.4	92.2	74.3	21.7	74.0	1.6	230	<5	2.24	38.7	15.0	5.6
4 days at 16°, 2 days at 20° C	10	5.7	88.9	76.8	21.2	72.7	1.4	202	5–7	2.28	33.7	12.2	5.7
2 days at 16°, 4 days at 20° C	11	6.3	88.5	76.5	21.2	72.8	1.6	199	7	2.28	33.0	12.2	5.8
4 days at 20°, 2 days at 12° C	12	5.9	89.8	79.3	21.4	73.0	1.5	186	10	2.31	33.2	12.7	6.1
2 days at 20°, 4 days at 12° C	13	6.2	90.2	96.8	21.2	73.6	1.5	218	5–7	2.31	36.7	14.4	6.2
4 days at 20°, 2 days at 16° C	14	6.8	89.3	78.5	21.2	72.7	1.6	210	5	2.31	33.5	12.5	6.1
2 days at 20°, 4 days at 16° C	15	5.9	89.9	87.8	21.5	72.9	1.8	200	<5	2.38	34.0	12.7	6.0

[1] Average of duplicate maltings.

Materials and Methods

The four barley varieties used were grown in 1939 and were the same as those for the experiment reported by Shands *et al.* (1941), namely (1) Oderbrucker (Wisconsin Pedigree 5-1), (2) Wisconsin Barbless (Wisconsin Pedigree 38), (3) Peatland, and (4) Chevron. Lots of the first two varieties came from Wisconsin farmers' fields in regular agricultural production. Small kernels were removed from these lots by cleaning and sizing equipment. Peatland and Chevron were grown in the yield trial plots on the West Hill Farm of the Wisconsin Agricultural Experiment Station. After threshing, these latter two varieties were subjected to cleaning by means of a Clipper mill. The barleys had the following kernel weights in milligrams, dry basis: 31.7, 31.2, 22.9, and 24.0, respectively, for the varieties as listed above. The barley nitrogen percentages in the same order were as follows: 2.02, 1.96, 2.06, and 2.24.

All samples were malted six days in a modified General Electric refrigerator equipped to provide positive circulation of humidified air and relatively accurate temperature control. Small screen-bottomed cans were used for steeping, germinating, and drying as described previously (1941). The same kiln or drier used in the earlier experiment was adjusted to dry the malts to approximately 5% moisture during a period of 31 hours. Thermostatically controlled electric heaters were used for the drying process.

A sample of each variety of barley was subdivided into 18 portions, each weighing 170 g, dry basis. Samples were placed into the steep tank at time intervals appropriate for absorbing 45% moisture at 16° C. The amounts of time required for steeping were 41, 43, 33, and 33 hours respectively, for the four barley varieties. The growing malts were stirred twice daily after being watered. The watering schedule was based on the expected respiration losses. The moisture content of the green malt, averaging slightly above 44%, indicated that the watering schedule was satisfactory.

Physical and Chemical Analysis

The 72 malts were analyzed for the following physical and chemical factors: steep and respiration loss, recovery, growth of acrospire, kernel weight, extract, diastatic power, conversion time, malt nitrogen, wort nitrogen, formol nitrogen, and pH of wort. The ratio of wort and formol nitrogen to malt nitrogen were calculated. Other factors studied, the results of which are not given in Table I, are: moisture content of steeped barley, green and dried malt, cleaning loss, and permanently soluble nitrogen. The analyses were conducted as described by Dickson and Burkhart (1942), and Shands *et al.* (1941).

Steep and Respiration Loss

Steep and respiration losses given in detail in the four sections of Table I ranged with the barley variety or malting method used, from as low as 3.1% to as high as 7.3%. Losses for Peatland and Chevron were greater than for Oderbrucker, those for Wisconsin Barbless being lowest. Averaging the four varieties, Method 4 (four days at 12° followed by two days at 16°C) gave the lowest loss, and the constant-temperature method of six days at 12°C gave a loss only slightly higher. The highest loss occurred at the constant temperature of 20°C. The results as averages for the four varieties are summarized in Table II and Figure 1A.

TABLE II

SUMMARY OF EFFECT OF DIFFERENT MALTING METHODS ON QUALITY FACTORS —AVERAGE OF FOUR VARIETIES

Malt quality factor	Malting time (days) at various temperatures, and method number														
	6 at 12°	6 at 16°	6 at 20°	4 at 12° 2 at 16°	2 at 12° 4 at 16°	4 at 12° 2 at 20°	2 at 12° 4 at 20°	4 at 16° 2 at 12°	2 at 16° 4 at 12°	4 at 16° 2 at 20°	2 at 16° 4 at 20°	4 at 20° 2 at 12°	2 at 20° 4 at 12°	4 at 20° 2 at 16°	2 at 20° 4 at 16°
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Steep and respiration loss, %	3.9	5.2	6.7	3.6	4.3	5.0	6.3	4.6	4.2	5.7	5.9	5.7	5.8	6.6	5.5
Recovery, %	93.2	90.5	88.2	92.8	91.4	91.0	88.8	92.3	92.6	89.4	89.2	89.9	90.9	89.6	90.7
Extract, dry basis, %	74.3	73.8	72.9	74.1	73.9	73.9	72.7	74.3	74.5	73.5	73.2	73.8	74.3	73.4	73.5
Color, *Lov*	1.3	1.5	1.6	1.3	1.4	1.4	1.7	1.6	1.5	1.3	1.5	1.4	1.4	1.6	1.6
Diastase, °*L*	162	172	164	159	163	165	163	182	179	165	159	154	173	166	169
Conversion time, *min*	8.3	6.4	7.5	7.6	8.0	8.5	5.5	5.8	5.0	7.9	8.5	10.9	8.9	5.8	4.8
Ratio of wort N to malt N, %	34.6	34.4	34.7	33.9	33.3	33.3	31.9	35.8	36.3	32.5	31.5	33.1	36.4	32.1	35.3
Ratio of formol N to malt N, %	13.4	13.4	11.6	13.4	13.6	12.4	11.5	14.2	14.4	12.0	11.9	12.8	14.5	12.1	13.1

Recovery

Recovery ranging from 87.3% to 93.8% was influenced by variety and method of malting. For Chevron and Peatland it was similar, being slightly less than Oderbrucker, which in turn was less than Wisconsin Barbless. Peatland and Chevron barleys were grown under field conditions different from those of Oderbrucker and Wisconsin Barbless, and for this reason indiscriminate varietal comparisons for recovery or other factors may be misleading. Averaging of values showed lowest recovery, 88.2, to result from the constant temperature of 20°C and highest, 93.2, from the constant temperature of 12°C. The next high recovery was with four days at 12° followed by two days at 16°C.

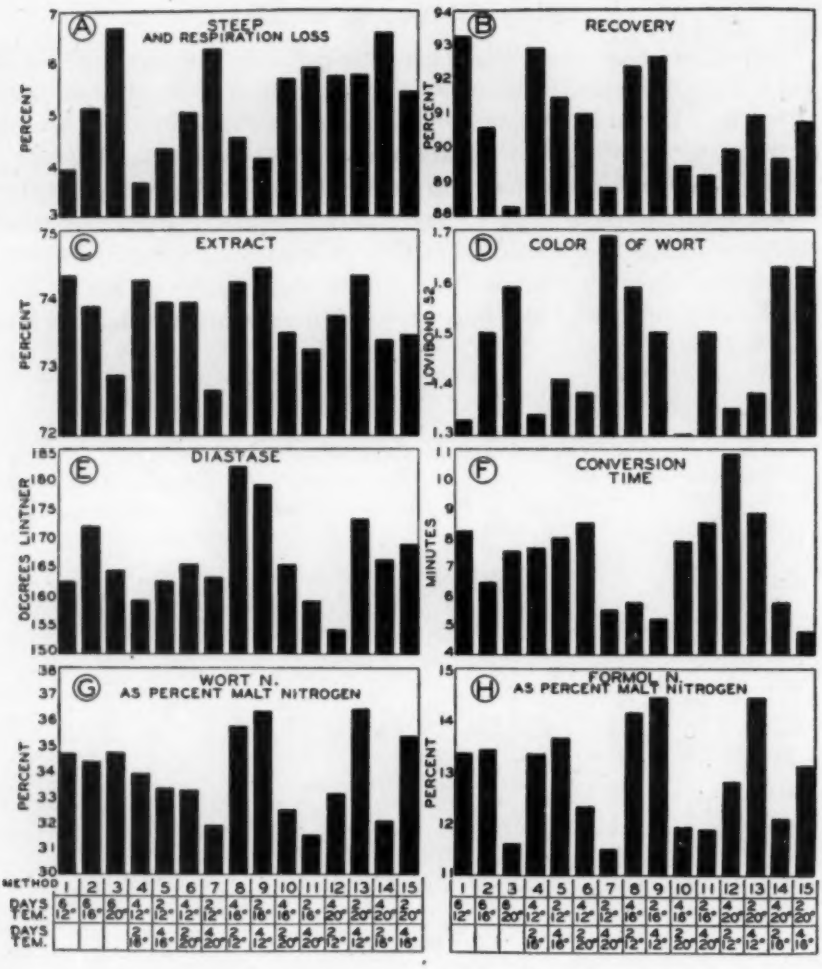

Fig. 1. Summary of the effect of different malting methods on quality factors.

Growth of Acrospire

Growth index of acrospire ranged from 71.5 to 99, being influenced by both barley variety and malting method. Method 13, with two days at 20° and four days at 12°C, gave the highest growth index. Why this method gave more growth than occurred with other methods is not understood.

Kernel Weight

Kernel weights varied slightly around two sets of means, one for Oderbrucker and Wisconsin Barbless, and another for Peatland and

Chevron. The method giving highest kernel weight involved, as in the case of recovery, the constant 12°C and, similarly, the lowest involved the constant 20°C. The methods involving change of time and temperature gave intermediate values.

Extract

Malt extract values varied over a fairly narrow range of 71.9 to 75.5. Oderbrucker extract was about 1½% greater than Wisconsin Barbless, while Chevron was about ½% lower than Peatland. Method 7, two days at 12° and four days at 20°C, gave the lowest average extract percentage, followed closely by the constant 20°C. Highest extract was obtained by Method 9, which was two days at 16° and four days at 12°C. This was followed closely by other methods as can be seen in Table II and Figure 1C.

Color

Wort color readings were low for the most part, varying from 1.1 to 1.9 (Lov. 52). Peatland and Chevron gave higher colored worts than Wisconsin Barbless and Oderbrucker. Method of malting also influenced color over a range of 1.3 to 1.7, as will be seen in Figure 1D.

Diastatic Power

Enzyme development as measured by diastatic power or conversion time was greatly influenced by both barley variety and malting methods. The range in degrees Lintner was from 109 for Wisconsin Barbless to 239 for Chevron. The largest differences were attributable to varietal response. Oderbrucker was higher in diastatic power than Wisconsin Barbless, and Chevron was higher than Peatland, comparisons which agree with previous studies. The method responsible for the highest average diastase value was No. 8, or four days at 16° and two days at 12°C. Table II and Figure 1E show that Method 8 was followed closely by No. 9; and the lowest diastase was produced by Method 12, or four days at 20°C and two days at 12°C.

Conversion Time

Several of the malts showed a conversion time of less than 5 minutes while one was 15 minutes. Wisconsin Barbless was slower in conversion time than the other varieties, and Peatland seemed slower than Chevron. In deriving average figures for conversion time in Table II, less than 5 minutes was arbitrarily calculated as 4 minutes, and 10–15 minutes was given the value of 12.5. There seems to be an association of high diastase and low conversion time, though by no means a perfect correlation. When conversion time was averaged for the four

varieties, Method 15, two days at 20° and four days at 16°C, showed the shortest time for conversion. The results are given graphically in Figure 1F.

Malt Nitrogen

Malt nitrogen appeared to be largely a varietal characteristic. Variation in amount caused by different methods was small and of doubtful significance. Wisconsin Barbless averaged slightly lower in nitrogen than Oderbrucker, a result which could be expected on the basis of the nitrogen content of the original barley. Chevron malts were higher in nitrogen than Peatland malts.

Wort Nitrogen

The ratio of wort nitrogen to malt nitrogen expressed as a percentage varied considerably. The range was from 25.8% to 40.0%, depending upon variety and malting method. Peatland had a higher wort nitrogen ratio than Chevron, and Oderbrucker was higher than Wisconsin Barbless. When varieties were averaged, the method of two days at 20° and four days at 12°C gave the highest ratio, while two days at 16° and four days at 20°C gave the lowest ratio. Method 9 also gave a high ratio of wort to malt nitrogen.

Formol Nitrogen

Formol nitrogen seemed to be associated with wort nitrogen. There was a range from 9.1% to 16.1%, depending upon variety and malting method. As in the case of the wort nitrogen ratio, two days at 20° and four days at 12°C gave the highest formol nitrogen ratio. Lowest formol nitrogen ratio was obtained in the reciprocal method, namely two days at 12° and four days at 20°C.

pH Reaction of Wort

The pH of the worts varied from 5.5 to 6.2. Varieties reacted similarly for the most part, while methods produced rather definite differences. Two days at 12° and four at 20°C, the method that gave lowest formol and second low wort nitrogen ratios, gave the lowest pH value. The high pH value was obtained with two days at 20° and four days at 12°C, which was the method giving highest formol and wort nitrogen ratios.

Summary and Discussion

Summaries of several of the malting factors used in this study are given in Table II and Figure 1. The values listed are averages of the four varieties for a particular method of malting. The data given in

Table I have been subjected to statistical treatment, and statements made in this paper have the benefit of this analysis. In interpretation and application of the results, several conditions must be kept in mind. First, the limiting of all methods to six days of malting necessarily prevented daily interval observations for sequence of chemical and physical changes. Second, all methods were limited to a single moisture during growth. It is therefore impossible to show the compensating effect of different moistures. In other words the experiments determined the combined influence of temperature change for the six-day malting period and do not show the progressive modification and chemical composition at different stages or intervals of growth. Only a six-day cross section of the malting process was available, and what would be applicable to six-day malts might not apply to four-, five-, or seven-day malts. In an earlier experiment where malts were grown for two-, four-, six-, and eight-day intervals, trends for various factors were available for interpreting the data.

Malting methods which gave low steep and respiration losses gave high values for recovery, extract, and formol nitrogen. Recovery percentages also closely paralleled those of extract and formol nitrogen, and less closely those of wort nitrogen. High extract was associated with high formol nitrogen ratios, and to a less extent with a high wort nitrogen ratio. There was a suggestion that high extract values and prolonged conversion times were related to low wort colors. More diastatic power usually accompanied higher wort and formol nitrogen ratios and shorter conversion time. Wort and formol nitrogen ratios responded somewhat similarly to different malting conditions. Restating some of these relationships, formol nitrogen was positively correlated with extract, recovery, wort nitrogen, and diastatic power, and negatively correlated with steep and respiration losses. Wort nitrogen ratios followed similar trends with diastase, extract, and recovery. Thus it will be seen that several important interrelationships existed in this group of malts produced under the limited conditions already described.

The use of different steep and malting moistures might serve to bring out malting responses that would otherwise be overlooked. With emphasis on caution in interpretation or application of the results described in this paper, a few methods either of constant or changed conditions may be compared. Methods 8 and 9 under experimental conditions produced malts that were apparently superior to other malts produced either by constant or changed conditions. Malts produced by using two days at 16° and four days at 12°C were high in recovery, extract, diastase, ratios of wort and formol nitrogen, low in steep and respiration loss, and low in conversion time, with color being accept-

able. In contrast, two days at 16° and four days at 20°C gave malts low in recovery, extract, diastase, ratios of wort and formol nitrogen, and high in steep and respiration loss, and high in conversion time, with an intermediate color. These results indicate that within the ranges used, reduction in temperature after germination has progressed for a short time might improve quality, and conversely a temperature rise after germination is well started might deplete quality.

The fact that inferior malt was produced by a particular method does not necessarily imply that all of its features are bad. By reducing growth time, for example from six to five days at 20°C, a more desirable malt from the standpoint of recovery and extract may be produced. Other modifications in malting method may likewise improve quality. Furthermore length of malting time might be of considerable importance in plant operation. If less than six days had been used, the above methods might have produced different relationships.

The results of this experiment, in general, point to the possibility of improving malt quality by relatively simple temperature change during the malting period. They also indicate the desirability of further experimentation in finding responses to additional changes that could be made in malting. If temperatures were gradually reduced or increased, malting factors might be expressed somewhat differently than under conditions of constant temperature. Moisture change during malting might also influence the expression of quality factors. Using different moisture levels out of steep in combination with different growth periods and different temperature schedules might offer approaches for the production of more desirable quality and at the same time maintain efficient plant operation. Since previous experimentation with the physiology of malting has netted sizable returns in improving quality, it seems logical that further experiments along the lines suggested might open avenues for still more improvement.

Acknowledgments

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EFFECTIVENESS OF DRY MILK SOLIDS [1] IN PREVENTING OVERBROMATION OF SOME BLEACHED HARD WINTER WHEAT FLOURS [2]

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In recent years considerable work has been done to show the buffering effect of dry milk solids towards excessive amounts of potassium bromate in the bread formula. Various investigators have reported somewhat similar dough-improving effects from the use of bleaching agents and from potassium bromate. Since it is a common practice to use various oxidizing agents when baking bleached flours, the following question has been raised: Will the use of dry milk solids prevent the deleterious effects resulting from a combination of excessive quantities of potassium bromate and bleaching agents? The present investigations were undertaken to study this problem.

Review of Literature

The present common practice of supplementing the baking formula with milk is relatively new to the commercial baker, although an ancient practice of the housewife. Since Sherman, Rouse, Allen, and Woods (1921) investigated milk in bread from a nutritive viewpoint there has been considerable attention given to the study of milk, particularly dry milk solids, in bread.

Studying the action of phosphatides in dough, Working (1928a) found that if 4% to 6% of dry milk solids were added to the bread formula an improvement could be obtained by the addition of both a phosphatide and an oxidizing agent.

St. John and Bailey (1929) demonstrated that the buffering action of dry milk solids was appreciable, as shown both by the initial hydrogen-ion concentration of the freshly mixed doughs and by the relative rate of change in pH of control and milk-containing doughs. They concluded that the greater stability of milk doughs might be the consequence of the slower change in hydrogen-ion concentration. Skovholt and Bailey (1932) observed greater improvements by adding malt to doughs containing dry milk solids than to milk-free doughs. Increased scores resulted from the use of Arkady in every case when

[1] The term "dry milk solids" refers to the product made by removal of the water and fat from milk and contains not over 1½% fat and not over 5% moisture.

[2] Contribution No. 78, Department of Milling Industry. Condensed from a thesis submitted to the graduate faculty, Kansas State College, in partial fulfillment of the requirements for the degree of Master of Science.

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used in doughs containing dry milk solids. The improvement obtained by the use of Arkady under these conditions was much greater with winter wheat flours than with spring wheat flours.

Larmour, Working, and Ofelt (1939), using hard winter wheat flours, observed that the addition of 6% dry milk solids to the bread formula made it possible to include sufficient potassium bromate to condition the flours of the highest bromate requirement without over-oxidizing those of very much lower requirement. Using 4% dry milk solids, Barmore, Finney, and McCluggage (1939) report the beneficial effect of milk and bromate when included in the baking formula. These studies were expanded to include additional crop season samples and have been reported by Finney and Barmore (1941). Ofelt and Larmour (1940) observed that dry milk solids, together with the appropriate amounts of bromate, produced increases in loaf volume and improvements in texture beyond what could have been obtained with optimum amounts of potassium bromate alone. They pointed out the commercial significance of the buffering effect of dry milk solids toward bromate as being a safeguard against the possibility of damaging flours that had already been brought close to their optimum "oxidation" condition by bleaching or by addition of other oxidizing agents. This work was confirmed by Eisenberg (1940) and by Harris and Bayfield (1941), who presented corroborative data.

Working (1928b) observed that bleaching of flour had the same or a similar effect in developing the dough made from that flour as the addition of oxidizing agents to the dough. Hanson (1932) found that double the amount of nitrogen trichloride required to effect a full bleach did not in any way impair the baking quality of the flour. He observed that the addition of potassium bromate to the full bleach and to the double-bleach treatments did not show any beneficial results.

Rich (1934) observed that the action of potassium bromate appeared to be similar to that of nitrogen trichloride but more pronounced. He suggested that the maturing effect of bromate and nitrogen trichloride seemed to be dependent on the protein content of the flour. He indicated that the maturing action of nitrogen trichloride depended primarily on some reaction with the germ content of the flour. The similar effect of bromate suggested the reaction to be one of oxidation.

Balls and Hale (1936) suggested that the alteration of flours by bleaching was due to a diminution of the proteolytic activity, brought about by the oxidation of the activator of the flour proteases.

Harris and Bayfield (1941) found that bread was improved by the addition of increasing amounts of Agene and Novadelox, but that it was impossible to equal the improvements produced by the addition of bromate. Because of these differences they concluded that "bro-

mate action" and "bleaching action" should not be used interchangeably as meaning the same thing.

Working (1928b) attributed the beneficial effects of small amounts of oxidizing agents to their action in rendering phosphatides more soluble in water. Geddes (1930) concluded that the response of a flour to bromate depended on the state of oxidation of the germ constituents. Geddes and Larmour (1933) found that as the quantity of bleach was increased there was a definite trend towards decreased bromate response.

Rich (1934) suggested that the action of flour improvers must be colloidal, as the small amounts used could not effect gross chemical changes. His investigation indicated that bromate acted on the germ and protein constituents. Jørgensen (1936) restated his arguments in which he discredited the phosphatide and "electrolyte" theories and suggested instead that bromate paralyzes the proteinases, or their activators, of the wheat flour.

It was suggested by Sullivan, Howe, and Schmalz (1936) that the water-soluble substance responsible for the deleterious effects of germ was glutathione. They suggested that oxidizing agents changed some of the S-H glutathione to the S-S form which was unable to activate the proteases.

Mohs (1940) attributed the effect of flour improvers to their influence on the physical phase of the flour constituents. He suggested that their main influence was concerned with changes of surface tension and consequently the regulation of swelling processes or enzyme activity.

It is evident from a study of the literature that no majority agreement has been reached as to the fundamental reason for the improvement in bread which results from the use of commercial bleaching agents, potassium bromate, and milk. It is also evident that the use of milk in bread-making improves the product and guards against failure resulting from accidental overbleaching of the flour or overactivity of the dough.

Materials and Methods

The wheat varieties selected for this investigation were Tenmarq, Turkey (Kansas No. 570), Chiefkan, and Nebred. As a result of growing conditions, the samples were higher in protein content than is normally desired for bakery flour production. This made test baking difficult, especially when using the milk formula which produced large volumes that were somewhat hard to replicate satisfactorily. Table I gives analytical data for these samples.

With the exception of Chiefkan, these wheats were pure varieties. Chiefkan, however, was known to contain approximately 15% of other

TABLE I

ANALYTICAL DATA FOR WHEAT AND FLOUR

Variety	Test weight	Wheat		Flour			
		Protein[1]	Ash[1]	Yield[2]	Protein[1]	Ash[1]	Absorption[1,3]
	lbs	%	%	%	%	%	%
Tenmarq	57.8	16.3	1.557	71.6	15.7	0.372	65.4
Turkey	59.2	15.4	1.621	70.1	14.5	0.384	61.5
Nebred	60.7	15.4	1.585	72.7	14.8	0.397	61.1
Chiefkan	60.6	16.5	1.430	71.7	15.8	0.379	56.7

[1] Moisture basis 15%.
[2] Total weight of flour on basis of dry cleaned wheat.
[3] Values for milk-free formula without bromate.

winter wheats of good baking quality, and was materially benefited by this admixture. The extent of the improvement brought about by the impurity in the Chiefkan is indicated in Table II.

TABLE II

THE EFFECT OF THE 15% IMPURITY GROWN WITH CHIEFKAN [1]

Sample	Wheat protein[2]	Loaf volume
	%	cc
Pure Chiefkan	15.6	830
Bearded wheat impurity	16.7	1250
Mixture	15.9	950

[1] The separation of Chiefkan from other wheats was made from bundles of unthreshed grain.
[2] Moisture basis 15%.

Ten-bushel samples of Tenmarq, Turkey, and Chiefkan were milled on the Kansas State College 65-barrel mill. These three wheats were grown under comparable conditions at Manhattan. Only a limited supply of Nebred was available; therefore it was milled on a Buhler experimental mill. The Nebred sample was made up from two lots of this variety, the larger proportions being grown at the Nebraska Agricultural Experiment Station, Lincoln, Nebraska. The experimental flours were placed in a refrigerated room (40°–45°F) immediately after milling to reduce natural aging and bleaching to a minimum.

The baking formula used in this investigation was identical with that used by Harris and Bayfield (1941), as follows:

	Grams
Flour	100.00
Sugar	6.00
Salt	1.50
Yeast	2.00
Shortening	3.00
Malt syrup (120° L)	0.25
Water (distilled)	As required
Dry milk solids	(0 or 6) as indicated
Potassium bromate	(0 to 0.007) as indicated

The addition of 6% of dry milk solids resulted in an increase of 4% in the absorption value. The absorption for each variety was held constant without regard to the bleaching treatment, although the absorption was increased with increasing increments of potassium bromate.

Doughs were mixed at 100 rpm in a Swanson-Working mixer, using a bowl containing two opposite pins. The doughs were all given an optimum mixing time, and were fermented and proofed at 86°F and 80% relative humidity.

Fermentation was as follows:

	Minutes
First punch after	105
Second punch after	50
Mold after additional	25
Total	180

A National "pup" sheeting roll was used for punching while the doughs were molded on a Thompson laboratory molder. The loaves were baked at 420°F for 25 minutes in a specially designed Despatch oven equipped with a rotating hearth. Loaf volumes were taken immediately upon removal of the loaves from the oven. All bakes were replicated on different days for loaf volume to agreement within 25 cc. The loaves were cut and graded for internal characteristics approximately 16 hours later. The baking laboratory was conditioned, the temperature being held between 76° and 78°F.

Agene was added to the flour in quantities equivalent to 3 g and 9 g per barrel, which is referred to as normal bleach and 3-normal bleach, respectively. Novadelox was added to the flour in quantities equivalent to 0.4 oz and 1.2 oz per barrel, which is also referred to as normal and 3-normal bleach. All bleached samples were stored for two weeks before baking in tightly closed cans in a laboratory held at 70°F.

To determine the effectiveness of the bleaching agents and the extent to which the flours were bleached, flour pigment determinations were run on all the flour samples. A Wilkens-Anderson K. W. S. Z. photometer was used in connection with the method described by Binnington and Geddes (1939).

Experimental Results

The efficiency of the various bleaching treatments in reducing the yellow color in the flour is shown in Table III. No evidence of gray color was noticed from the heaviest bleach used. Nebred, a naturally highly pigmented variety, and Turkey, containing the second-highest amount of pigment, bleached readily with either of the bleaches employed.

TABLE III

AMOUNT OF FLOUR PIGMENT IN FOUR WINTER WHEAT FLOURS

Flour treatment	Tenmarq	Turkey	Nebred	Chiefkan
	ppm	*ppm*	*ppm*	*ppm*
None	1.74	2.49	2.95	1.73
Agene, 3 g per bbl	0.61	0.80	0.86	0.64
Agene, 9 g per bbl	0.33	0.49	0.41	0.43
Novadelox, 0.4 oz per bbl	0.90	1.21	1.56	0.75
Novadelox, 1.2 oz per bbl	0.26	0.46	0.54	0.36

Table IV presents loaf-volume data obtained from bread baked in these investigations. To reduce tabular material the bread scores for grain, texture, and crumb color have been omitted. It is interesting to note, however, that the optimum or best grain and crumb color scores normally were obtained at a bromate level 1 to 2 mg less than that required to obtain the largest or optimum loaf volumes, which are shown in bold-face type. Texture scores usually were improved by the use of milk in the formula. Crumb color was influenced by the amount of flour pigment remaining after bleaching.

Loaf-volume data are also shown graphically in Figures 1 (Agene-bleached flours) and 2 (Novadelox-bleached flours). These figures illustrate clearly the "buffering effect" of dry milk solids when relatively large amounts of bromate are used with or without bleaching. The figures also illustrate that the four varieties of wheat included in this experiment respond differently to the treatments imposed upon them.

It was observed that the flour pigment values tended to be reflected in the crumb color scores. It was desired to find whether the baking process itself might not also cause the removal of some color. Using unbleached Turkey flour, a number of bakes were made and the pigment content of the dried crumb determined. The baking treatments used and the results of the pigment determinations are presented in Table V. From these data it is evident that baking reduces the pigment content to some extent and that of the three factors varied, mixing time was the most effective.

Discussion

Examination of the data indicates clearly that the variety of wheat from which the flour was milled played an important part in the results obtained and in the conclusions which may be drawn from the investigations. The data show that the different varieties responded differently to bleaching treatment, to added bromate, and to added milk solids. In considering the results, the differences in amount of flour

TABLE IV

EFFECT OF BLEACHING AGENTS, POTASSIUM BROMATE, AND DRY MILK SOLIDS UPON THE LOAF VOLUME OF FOUR WINTER WHEAT FLOURS

$KBrO_3$ added mg	Loaf volume in cubic centimeters for each variety[1]							
	No milk solids				6% dry milk solids			
	Tenmarq	Turkey	Nebred	Chiefkan	Tenmarq	Turkey	Nebred	Chiefkan
NO FLOUR TREATMENT								
0	835	665	720	610	870	760	920	645
1	1060	920	910	840	—	—	—	—
2	1103	1053	**960**	960	—	—	—	—
3	**1107**	**1075**	910	**1000**	1188	—	1135	940
4	1007	1023	—	990	**1255**	1075	**1178**	960
5	—	—	—	—	1241	1140	1170	965
6	—	—	—	—	1237	**1146**	1153	980
7	—	—	—	—	—	1140	—	**998**
AGENE, 3 G PER BBL								
0	892	755	827	698	958	855	998	713
1	1123	985	**905**	873	—	—	—	—
2	**1145**	**996**	885	997	—	—	—	—
3	1020	958	820	**1035**	1220	—	**1090**	—
4	—	873	—	963	**1223**	1140	1075	970
5	—	—	—	—	—	1148	—	988
6	—	—	—	—	1217	—	1018	—
7	—	—	—	—	—	**1155**	—	**1013**
AGENE, 8 G PER BBL								
0	938	800	843	725	1028	898	1018	735
1	**1103**	980	**920**	905	—	—	—	—
2	1097	**1008**	863	**1000**	—	—	—	—
3	1012	993	790	980	**1263**	—	**1060**	—
4	—	880	—	963	1256	**1160**	1025	963
5	—	—	—	—	—	1135	—	990
6	—	—	—	—	1218	—	968	—
7	—	—	—	—	—	1105	—	**1008**
NOVADELOX, 0.4 OZ PER BBL								
0	900	713	763	658	978	805	935	683
1	1088	920	923	880	—	—	—	—
2	**1168**	**1043**	**937**	1000	—	—	—	—
3	1058	1020	885	**1020**	1190	—	1065	—
4	—	888	—	965	**1263**	1165	**1110**	978
5	—	—	—	—	—	**1180**	—	**1033**
6	—	—	—	—	1223	—	1016	—
7	—	—	—	—	—	1120	—	1015
NOVADELOX, 1.2 OZ PER BBL								
0	912	730	817	670	983	818	973	693
1	1120	913	947	880	—	—	—	—
2	**1163**	**1065**	**955**	**1013**	—	—	—	—
3	1047	1010	885	1013	1228	—	1125	—
4	—	910	—	977	**1280**	1143	**1138**	968
5	—	—	—	—	—	**1192**	—	**1010**
6	—	—	—	—	1253	—	1055	—
7	—	—	—	—	—	1165	—	985

[1] Bold-face type refers to optimum bromate level for maximum loaf volume.

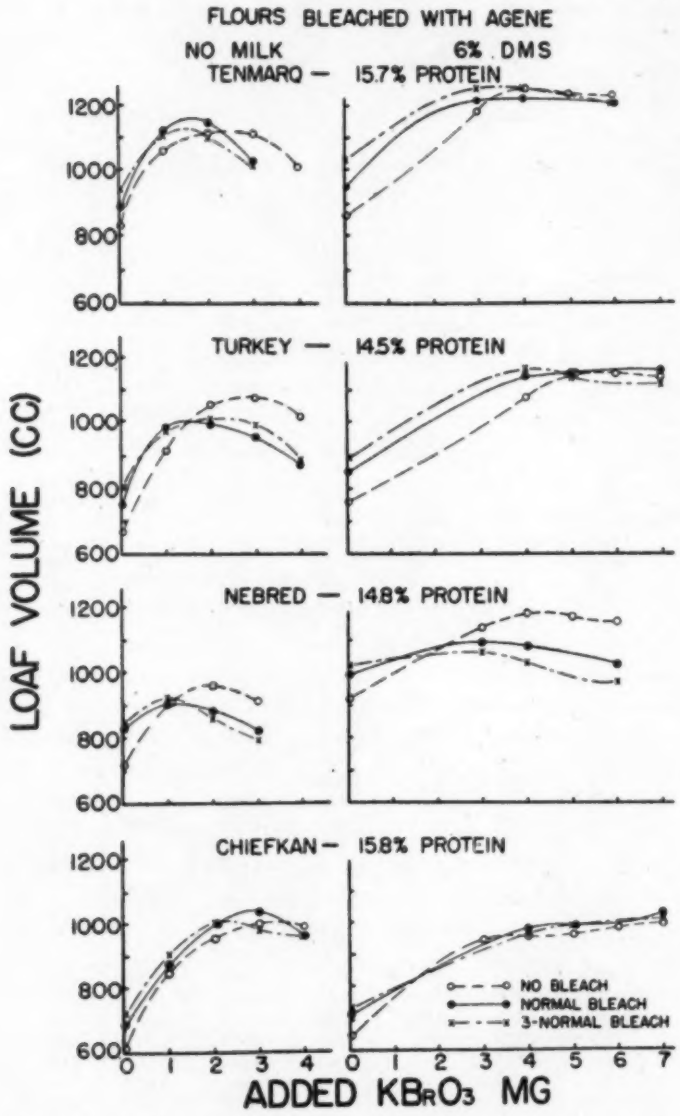

Fig. 1. The effect of potassium bromate upon the loaf volume of flours bleached with Agene.

protein must also be remembered. In this respect Tenmarq and Chiefkan were comparable, but they possessed a decided advantage over both Turkey and Nebred, which were approximately 1% lower in flour protein. Nebred had a slight advantage over Turkey but these two varieties may logically be compared.

Without potassium bromate, all flours responded favorably to

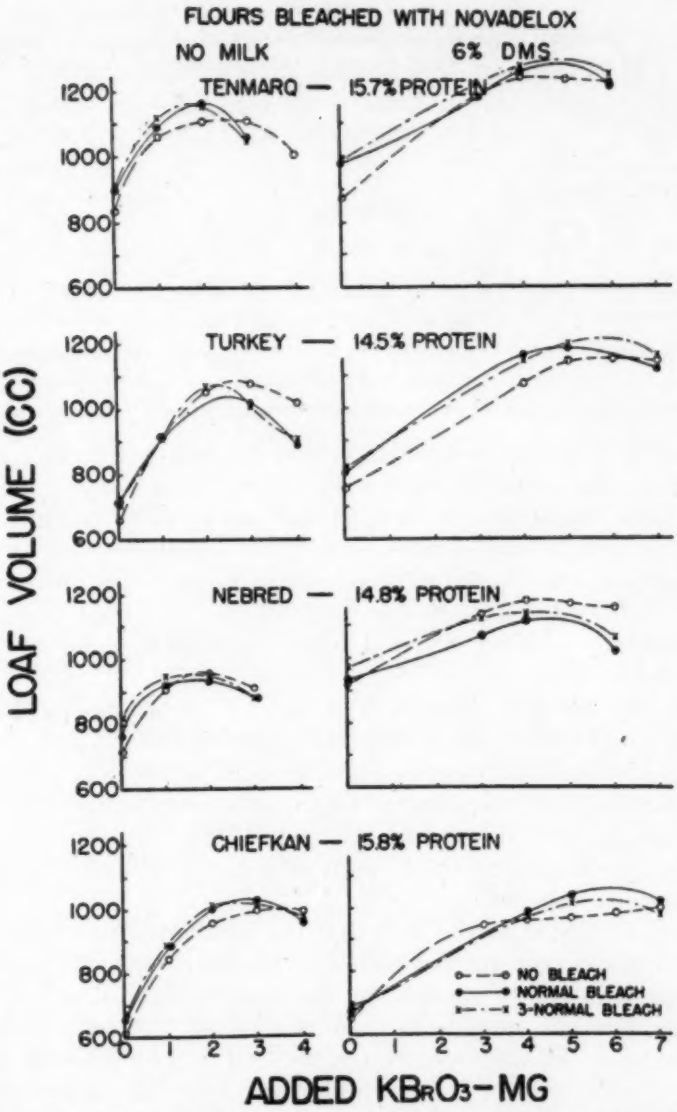

Fig. 2. The effect of potassium bromate upon the loaf volume of flours bleached with Novadelox.

bleaching, and the addition of 6% of dry milk solids to the formula produced still further improvement in the baked bread. Without bromate and without dry milk solids the Agene-bleached flours gave results superior to the Novadelox treatments. With added milk and no bromate, the Agene-treated Turkey was superior to the Novadelox-treated, but little apparent difference existed between the bleaching treatments with other varieties.

TABLE V

FLOUR PIGMENT CONTENT OF DRIED BREAD CRUMBS OBTAINED FROM UNBLEACHED TURKEY FLOUR BAKED IN SEVERAL WAYS

Treatment	$KBrO_3$ used	Flour pigment
	mg	*ppm*
Flour before baking		2.49
Normal bake [1]	0	1.26
	1	1.40
	3	1.39
	5	1.48
	10	1.41
Fermentation time—1 hour	0	1.13
Fermentation time—5 hours	0	1.10
Mixing time—1 min	0	1.40
Mixing time—5 min	0	0.88

[1] Normal bake used 3-minute mixing time and 3-hour fermentation time.

Without bleaching treatment, Nebred responded very favorably to added dry milk solids, Chiefkan very little. It was evident that Nebred differed fundamentally from Turkey in respect to "milk requirement" for best baking performance. This sample of Nebred when baked without milk proved to be inferior to Turkey when the bromate requirement needed for optimum loaf volumes was used. Tenmarq responded favorably to dry milk solids without bromate but to a smaller extent than Nebred.

Generally speaking, increasing the amount of bleach produced an increase in loaf volume unless excessive amounts of bromate were used. The data indicate that Agene is somewhat more severe than Novadelox in its bleaching and maturing actions. This corroborates the earlier results obtained by Harris and Bayfield (1941). In most cases the bleaching agent reduced the amount of bromate necessary to attain maximum loaf volumes.

It was evident from the data that the addition of dry milk solids conferred considerable tolerance against the adverse effects of excessive quantities of bromate, whether the flours were bleached or not. An exception, however, must be made in the case of Nebred, which after bleaching gave definitely poorer results with or without milk at the optimum bromate level for the unbleached flour. It is unfortunate that the optimum bromate level was not attained in the Agene-treated Nebred series because of exhausting the flour supply. However, it was considered that a 2-mg bromate-milk bake would not have altered materially the general conclusions regarding this variety.

Compared with Tenmarq, the Chiefkan variety proved to be definitely inferior. Chiefkan proved to be remarkably lacking in ability to respond to the treatments given. All evidence to date shows that it possesses poor-quality gluten when compared to standard varieties such as Turkey or Tenmarq.

These investigations were undertaken to determine whether the use of dry milk solids would reduce the possibility of damage resulting from the overbromating of bleached hard winter wheat flour doughs. This is a question of considerable practical importance to the baking industry. The results obtained warrant an affirmative answer to the question.

Summary

A study was made with four winter wheat flours to ascertain the effectiveness of dry milk solids in preventing damage from overbromation in the baking of flour previously bleached with Agene or Novadelox. In addition, the combined as well as the individual effects of bleaching agents and potassium bromate were studied.

All samples, bleached or unbleached, were baked with or without 6% of dry milk solids. The flours were baked with amounts of potassium bromate ranging from 0 to 7 mg per 100 g of flour. Agene and Novadelox bleaching agents were used in normal and in 3-normal quantities.

Chiefkan showed little improvement from the addition of dry milk solids, and proved inferior to the other three varieties in baking quality. Nebred flour before and after bleaching proved to be very sensitive to added potassium bromate. Nebred benefited more than the other varieties from the addition of dry milk solids, although when bleached it gained little tolerance to bromation when milk was added. The superior baking quality of Tenmarq and of Turkey flours was again demonstrated.

Unbleached samples of Nebred and Turkey flours contained more pigment than samples of Tenmarq or Chiefkan. Normal treatment with Agene was more effective in the removal of color than the same treatment with Novadelox. The 3-normal treatment with either bleach reduced all varieties to approximately the same pigment content.

The four varieties studied varied in their response to added bromate, to dry milk solids, and to bleaching agent used.

The results obtained showed that the presence of 6% of dry milk solids in doughs reduced the possibility of damage from excessive amounts of bromate used in baking unbleached or bleached samples of flour. The inclusion of dry milk solids increased the loaf volume and improved the crust and crumb color, grain, and texture. Bread was appreciably improved by bleaching the flour but greater improvement resulted from the use of potassium bromate in the dough than from bleaching the flour.

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BAKING TESTS AND THE EVALUATION OF NEW WHEATS [1]

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In the search for improved wheats a continuous stream of new varieties or strains flows to the cereal chemist for tests as to quality. These newer sorts are grown on small plots of land and frequently barely enough wheat is available for a small milling sample and a strictly limited number of bakings. The term "quality" has long been used in a broad sense, and without any standardized meaning. For the purposes of this discussion, however, "high or good quality" wheat will refer to a wheat which possesses properties making it well adapted for the market purposes of the class of wheat to which it belongs. The data reported at this time pertain to Kansas-grown wheats, and accordingly the quality designations will be used with particular reference to suitability for the market normally drawing a major part of its supplies from Kansas.

The bulk of Kansas-grown wheat consists of hard red winter varieties. From the standpoint of milling and baking qualities, the variety Turkey has, over the past several decades, proved widely acceptable as a strong bread wheat, and may be referred to as possessing good quality. Furthermore, other varieties possessing such properties or characteristics that they may be readily substituted for Turkey may also be considered as of good quality.

It must not be inferred that Turkey wheat, because of its excellent reputation for good quality, is the "perfect wheat" and may not be improved upon, because a wheat with Turkey qualities but without some of its deficiencies would undoubtedly find still wider acceptance by the trade. Turkey would be improved from a quality standpoint if it possessed heavier test weight per bushel with a consequent improvement in flour-yielding capacity, a somewhat lower amount of flour pigments, and a lower bromate requirement. Until new varieties with these advantages are produced, it is probable that wheat-quality improvement programs will continue. Notwithstanding room for improvements, Turkey does afford an acceptable standard of excellence in milling and baking quality. A new wheat possessing the properties of Turkey would readily find acceptance by millers using Southwestern wheats, and it is when new wheats differing widely from the accepted Turkey standards are distributed that complaints arise. To keep such

[1] Contribution No. 79, Department of Milling Industry.

complaints to a minimum and thereby maintain highest values to the grower, the use of the general principles outlined by Geddes (1941) is desirable for standardizing milling and baking qualities in new wheats distributed as a result of the efforts of the plant breeders.

Geddes (1941), in discussing the choice of new wheat varieties, states that: "The new variety should give satisfactory, and preferably optimum results in milling and baking when processed in the same manner as the 'standard variety' of the same wheat class. In this connection it is important to note that definite 'inferiority' in any one important quality characteristic is sufficient to render the variety unsuitable for release, even though it may be regarded as superior to the 'standard' variety in other particulars. Thus, higher flour-yielding ability cannot offset inferior baking properties, or good loaf volume, or undesirable dough characteristics."

Recently, in discussing baking results, Bayfield, Working, and Harris (1941) have commented on the need of care in choice of test procedures used in evaluating varieties so that varieties well adapted to consumer use may be selected. These workers found that two different baking methods tended to rank the varieties in different orders. More extensive data have been presented by Finney and Barmore (1941). This is not at all surprising, because any given baking procedure and formula will deviate more or less from the optimum for different samples. It is hardly conceivable that a baking technique will ever be found that is optimum for all samples because different varieties may be expected to respond in varying degrees to ingredients used in the baking formula and to physical operations involved. Fortunately it appears that some varieties possess the highly desirable capacity to give good baking results over a relatively wide range of baking conditions. Such varieties gain wide commercial acceptance because their flours are readily, and without specialized treatment, adapted to the different requirements of the bread-baking industry.

At this point it might be well briefly to emphasize the more important points to be considered in choosing a good baking test procedure. Today there appear to be two general philosophies governing the thinking of cereal chemists engaged in test baking. One is based on the development of methods whereby flours of different qualities and characteristics are made to give essentially the same baking results. This involves the adaptation of the baking test to the flour. A cereal chemist successful along these lines finds wide use of his skill as a "trouble shooter" in the baking industry. The second philosophy deals with methods whereby the flours are subjected to one or more uniform testing procedures in order to observe how well the flour fits into the requirements of a particular use. In this case a flour or wheat

variety must fit the testing procedure in order to be satisfactory. The authors believe that the latter general philosophy should govern in the variety testing program so as to eliminate undesirable wheats *before* they attain general distribution among the growers. The application of the former general philosophy to the variety testing program, it seems, will tend toward lack of uniformity in the quality of wheats grown in a given section of the country and cause much confusion for the milling and baking trades, which prefer, above all things, to produce a uniform product from day to day and throughout the year.

The baking test is probably the most important single test used in evaluating bread wheats. What are some of the requirements of a good baking test for *variety testing* work? The most important feature of any such baking test should be its tendency to rank or rate wheats in the order of their commercial acceptability. Unless the method employed will do this, it fails to achieve the major purpose of testing the samples. A second desired feature of almost equal importance would be that the method give as large a spread as possible between commercially desirable and undesirable varieties. This wide spread in results is needed so that differences between samples will not be due to errors or variations inherent in the baking test. A third feature is that the baking procedure should be practical and susceptible to "mass production" methods. In variety testing work large numbers of samples are customary, and intricate complicated testing procedures are not practical. Finally the formula should be as simple as it can be made because, as additional ingredients are included in the formula, variables are also added. There is always the possibility that such added ingredients may serve to cover up inherent weaknesses in a variety.

Numerous reports have been published dealing with the evaluation of baking qualities of new and old varieties of wheats. In spite of these reported results additional work is continually in progress as new methods and procedures necessitate the re-assessing of old and new wheats in the light of the improved techniques. The new wheats produced by the plant breeder, who often crosses rather diverse wheat types, introduce many new problems. These new wheats, as a result of their parentage, may frequently differ widely in characteristics such as mixing time, absorption, milling properties, flour granulation, bromate requirement, milk requirement, and so forth. These wide differences make the problem of finding a "perfect" or universal baking method adapted to all wheats increasingly unlikely. It is questionable if any formula and technique can give optimum bread results over the range of baking qualities encountered within one class of wheats, and it seems rather improbable that a given method can even be considered

as giving "optimum" baking results over the entire protein range within one variety.

The method which aims at producing optimum bread over a wide range in flour properties requires a high degree of skill based upon years of experience. Such a "skilled" baking method would entail varying formulas, fermentation times and temperatures, dough handling and other features of the baking process to suit each sample, and would not meet the requirements of the cereal chemist who for many years has striven diligently for more science and exactness in baking. While the application of the optimum principle to baking would give information as to requirements for making good bread from any flour, it is very doubtful if such information is needed for new varieties still in the hands of the wheat breeder, because the industry cannot readily change commercial bread making to suit a wide range in flour properties. The baker prefers flour that fits into the baking methods current in his shop. The miller therefore must buy wheat which will make the desired type of flour. To fit market needs and provide a good market for the wheat grower, it is therefore imperative that the plant breeder produce wheats which will find ready use by miller and baker. In this scheme of things the cereal chemist needs baking procedures which will accurately eliminate the unwanted varieties.

It has already been indicated that flour milled from Turkey wheat finds a ready market, and that in spite of its faults this variety may be used as a standard or base for comparison against which new varieties can be rated. Varieties which respond similarly to Turkey may be expected to fit the requirements of markets which now use Turkey flours. Varieties which do not respond similarly to Turkey may be suited to bread production but they will not be equally suited to the market needs. Good bread can be made from some soft winter wheat flours, yet this does not make these flours equally useful in a market accustomed to hard red winter wheat flour.

Recently, Parker and Bayfield (1941) reported upon a collaborative preference rating of quality for Turkey, Tenmarq, Blackhull, and Chiefkan wheats or flours milled from these wheats. Twenty-three laboratories ranked either Turkey or Tenmarq first, two preferred Blackhull as first choice, and one laboratory indicated Turkey or Tenmarq equal to Blackhull in first place, depending upon anticipated use of the flours. Chiefkan was a very poor fourth in order of preferences. The four wheats were grown under comparable conditions in acre plots. As the baking methods employed by these collaborators differed widely, the agreement in final opinions was surprisingly good. This study indicates that there should be no question as to the suitability of Turkey as a standard of comparison. Tenmarq, with a

lower pigment content and lower bromate requirement, may be considered superior to Turkey in these respects and its equal otherwise as a bread wheat.

The 1938 crop studies of Larmour, Working, and Ofelt (1939, 1940) and those for 1939 of Bayfield, Working, and Harris (1941) show that some varieties of hard red winter wheat are relatively poorer for bread production than some soft winter wheats. These studies in the present instance have been continued on the 1940 crop by the authors.

Materials and Methods

Small lots of grain of pure varieties were obtained [2] from widely scattered tests over the state of Kansas. These samples varied considerably in protein content as they originated from some 43 different locations. Hard red winter varieties included were Turkey, Tenmarq, Kanred, Cheyenne, Nebred, Early Blackhull, Blackhull, Chiefkan, Iobred, Iobred Selection, Pawnee (Kawvale x Tenmarq), and Commanche (Oro x Tenmarq); whereas Kawvale (semihard), Michigan Wonder, and Clarkan represented the soft red winter varieties.

After all unsound samples as well as those weighing less than 53 pounds per bushel were discarded, the protein content of each was determined. The samples were then composited according to variety so as to give, at different protein levels, composites which were sufficiently large for milling and baking tests. In making the required wheat mixes the following wheat protein levels were desired:

Below 10%	14.6–16.0%
10.1–11.5%	16.1–17.5%
11.6–13.0%	17.6% and above
13.1–14.5%	

Each variety was not grown at all locations and it was not possible to have all protein levels represented in each variety. By blending the samples on a protein basis without regard to location it was hoped to overcome the effect of environmental growing conditions. The composited samples were thoroughly mixed and then milled on a Buhler mill. The resulting straight-grade unbleached flours were analyzed, stored at room temperature in tightly covered cans for six weeks, and then removed to a refrigerated room until the baking tests were completed.

Analytical and baking procedures used in this study were similar to those given earlier by West and Bayfield (1942). In the present study four different formulas were used, and the given average loaf-volume scores resulted from two or more single loaves, each baked on

[2] The authors acknowledge their appreciation to A. L. Clapp of the Department of Agronomy for supplying these samples.

different days. The individual bakes were replicated until loaves checking within 20 cc were obtained. All samples were baked with Formula 1 (referred to as "rich" formula) and Formula 2 (malt-phosphate-bromate or MPB formula) while four varieties—Turkey, Tenmarq, Blackhull, and Chiefkan—were also baked with Formula 3 (modified MPB) and Formula 4 (modified rich formula). Optimum mixing times were used throughout. The composition of these formulas is given in Table I.

TABLE I

FORMULA INGREDIENTS

Ingredient (per 100 g flour)	Formula			
	1	2	3	4
	g	g	g	g
Flour [1]	100	100	100	100
Sugar	6	5	5	6
Salt	1.5	1	1	1
Yeast	2	3	2	3
Dry milk solids (DMS) [2]	6	—	—	6
Shortening	3	—	—	3
Malt syrup—120° L	0.25	—	—	0.25
Malt syrup—200° L	—	0.30	0.30	—
$NH_4H_2PO_4$	—	0.10	0.10	—
$KBrO_3$	0.004	0.001	0.002	0.004
Water	As needed	As needed	As needed	As needed

[1] Weighed out on 15% moisture basis.
[2] Containing not over 1½% fat and not over 5% moisture.

With the exceptions that 6% instead of 4% dry milk solids and 4 mg instead of optimum bromate were used, Formula 1 (rich formula) was identical with that used extensively by K. F. Finney in the Hard Winter Wheat Quality Laboratory of the U. S. Department of Agriculture at the Kansas Agricultural Experiment Station. Formula 2 (MPB), the malt-phosphate-bromate formula, is used extensively by spring wheat investigators. Formula 4 (modified rich) was the formula employed by Bayfield, Working, and Harris (1941) on Kansas-grown 1939 crop samples. Formula 3 (modified MPB) was included to study the effect of using less yeast and more bromate in the MPB formula, which, it was thought, might penalize varieties with a bromate requirement higher than 1 mg.

Results and Discussion

Table II presents both analytical and baking data for the different variety samples. These data give, among other things, information regarding the possible usefulness of the MPB formula for hard red winter wheats. The wheats provide a wide range of protein contents,

as well as widely differing baking qualities. It was desired to find whether this formula would give essentially linear relationships between loaf volume and protein content, particularly with varieties such as Blackhull or Turkey, which are commonly considered as possessing high bromate requirements. Recently Larmour (1941) has reported favorable results from the use of this formula when used on hard red winter wheats. Bayfield (1941) by using one-half the stipulated quantities of malt, phosphate, and bromate in this formula obtained satisfactory results in testing flours of soft winter wheat varieties. Bayfield and Shiple (1937) concluded that the use of the full amounts of malt, phosphate, and bromate was too severe for normal strength flours of soft winter wheat.

Results in Table II indicate that the same flour when baked by different formulas gave different loaf volumes. Some samples gave relatively larger or smaller volumes with certain formulas than with others. This means that the relative rankings of the varieties tend to change with baking formula as a result of differences in response to formula ingredients. As all varieties included in this study are not equally desirable from a utility or industrial standpoint, it obviously is essential that the proper formula be chosen; otherwise there is the danger that undesirable varieties will be awarded a higher ranking than they merit.

The varieties Turkey, Blackhull, Tenmarq, and Chiefkan were baked by all four formulas, preliminary to baking the other varieties, in order to observe whether Formula 3 might not prove superior to Formula 2 and whether Formula 4 would give similar or better results than Formula 1. Figure 1 shows the regression lines of loaf volume on protein content of flour when the above-mentioned varieties were baked by all four formulas. All four formulas indicated Chiefkan to be definitely of poor baking quality, thus confirming results obtained by Larmour, Working, and Ofelt (1939) and Bayfield, Working, and Harris (1941) on the 1938 and 1939 crops respectively. With all four formulas, Turkey gave satisfactory results, an indication that this variety possesses the very desirable characteristic of being reasonably tolerant to formula changes. However, the relative behavior of Turkey at different protein levels is changed by substituting Formula 4 in place of Formula 1.

The relative positions of Tenmarq and Blackhull with the different formulas are interesting and of considerable importance. It has already been indicated that a very large proportion of the trade prefers Tenmarq to Blackhull. The formulas containing milk and shortening indicate Blackhull as superior to Tenmarq. It was thought that possibly the use of 4 mg of $KBrO_3$ was depressing the baking performance of the Tenmarq. However, when the Tenmarq series was

TABLE II

ANALYTICAL AND BAKING DATA

Serial No.	Test weight[1]	Flour yield[2]	Wheat protein[2]	Flour ash[3]	Flour protein[3]	Absorption[3,4]	Loaf volume and mixing time[5] with formula 1		2		3	4
							min	cc	min	cc	cc	cc
	lbs	%	%	%	%	%						
TURKEY CI 1558												
829	59.0	70.1	9.0	0.38	8.2	55.3	4¼	673	4	590	548	593
830	58.8	69.0	10.5	0.39	9.3	56.1	4	773	3¾	663	618	693
831	59.0	70.3	11.3	0.40	10.2	56.8	3¾	835	3½	695	680	780
832	58.3	71.7	13.1	0.41	11.8	58.5	3½	944	3¼	825	813	900
833	57.0	71.4	14.3	0.44	13.3	60.0	3¼	988	3¼	843	873	942
834	54.2	70.7	16.8	0.45	15.5	63.7	3¾	1173	3¾	1078	1120	1178
TENMARQ CI 6936												
823	57.3	72.2	8.8	0.40	7.9	54.6	4¾	613	4½	558	513	583
824	59.3	73.3	10.4	0.39	9.3	57.2	4¼	677	4¼	615	558	643
825	58.5	73.3	11.6	0.37	10.8	58.2	3¾	780	4	707	668	755
826	58.7	72.9	12.8	0.38	11.8	60.1	3¾	838	3½	773	708	828
827	57.6	71.9	14.1	0.36	13.3	61.0	3¼	893	3¼	833	790	853
828	55.0	71.6	15.9	0.43	15.3	64.0	3¾	1118	3½	1045	965	1000
BLACKHULL CI 6251												
764	60.6	70.0	9.4	0.35	8.4	52.5	3¼	695	3¼	590	563	698
765	62.2	70.4	10.4	0.35	9.1	53.3	2½	753	3	613	608	720
766	62.0	70.5	11.8	0.35	10.5	54.1	2¾	825	2¼	693	708	813
767	60.3	70.0	13.1	0.36	11.8	55.1	2⅝	913	2¼	763	808	873
768	60.2	72.3	14.1	0.38	13.1	57.2	2½	940	2¼	795	868	890
769	57.3	70.6	15.9	0.41	15.1	59.0	2¾	1110	2¼	980	1105	1068
770	58.3	70.2	18.1	0.42	17.4	62.7	2⅝	1193	2¼	1085	—	1240
CHIEFKAN CI 11754												
780	62.2	74.0	9.7	0.42	8.7	54.4	2¼	640	2	558	563	593
781	62.0	74.7	10.4	0.41	9.3	55.5	2½	648	2	595	585	608
782	61.5	74.9	11.9	0.37	10.7	56.2	2⅜	720	1¾	662	650	698
783	61.6	74.4	12.9	0.37	11.6	58.5	2⅜	735	1¾	683	675	713
784	60.2	72.2	14.4	0.39	13.1	59.3	2	785	1¾	758	770	737
785	60.1	72.7	14.9	0.43	14.6	61.1	2	840	1¾	763	763	793
KANRED CI 5146												
797	59.4	74.8	9.6	0.36	8.6	55.0	3⅞	698	3¾	585	—	—
798	58.0	71.4	10.1	0.36	8.9	55.8	3¾	708	3¾	645	—	—
799	59.3	75.0	11.6	0.36	10.4	56.3	3½	785	3¼	685	—	—
800	57.6	73.8	13.2	0.38	12.0	57.1	3⅛	903	2¾	810	—	—
801	57.8	73.5	14.5	0.41	13.3	58.8	2½	937	2¼	823	—	—
802	56.2	72.4	16.3	0.43	15.5	60.6	2¼	998	2¼	907	—	—
803	55.0	74.3	18.0	0.46	17.4	64.8	2¾	1110	2¾	985	—	—
EARLY BLACKHULL CI 8856												
771	62.2	70.8	11.6	0.35	10.9	52.7	2⅝	798	2¼	680	—	—
772	61.8	71.4	13.2	0.36	12.4	54.8	2⅛	845	1¾	765	—	—
773	61.2	72.9	13.5	0.35	12.7	55.8	2	840	1½	720	—	—
774	60.9	72.7	15.4	0.37	14.7	58.6	2¼	935	1¾	820	—	—
CHEYENNE CI 8885												
775	60.5	73.9	10.3	0.37	9.2	55.2	6	685	5¼	608	—	—
776	59.2	70.8	11.6	0.37	10.4	57.2	5¾	743	4¾	682	—	—
777	57.2	72.7	12.3	0.40	11.2	58.1	5¼	820	3¾	705	—	—
778	59.9	75.6	14.4	0.44	13.2	59.5	4½	883	3¾	775	—	—
779	57.9	74.2	15.8	0.44	14.8	64.4	5½	953	4½	828	—	—
779A	56.6	74.8	16.8	0.50	15.6	65.2	5½	1010	4½	975	—	—

TABLE II—*Continued*

Serial No.	Test weight[1]	Flour yield[2]	Wheat protein[2]	Flour ash[2]	Flour protein[2]	Absorption[3,4]	Loaf volume and mixing time[5] with formula					
							1		2		3	4
	lbs	%	%	%	%	%	*min*	*cc*	*min*	*cc*	*cc*	*cc*
					IOBRED CI 6934							
791	62.2	74.0	10.8	0.34	9.6	54.4	4	703	3½	618	—	—
792	61.9	72.5	12.6	0.32	11.3	55.4	3½	830	2¾	718	—	—
793	61.2	75.5	14.0	0.34	13.0	56.5	3	953	2½	845	—	—
					IOBRED SELECTION CI 11997							
794	62.2	75.7	10.6	0.34	9.7	53.5	4½	720	3½	605	—	—
795	59.7	71.4	12.8	0.33	11.1	54.4	3¼	868	2¾	740	—	—
796	61.1	72.8	14.4	0.32	13.4	57.2	3	990	2½	868	—	—
					PAWNEE CI 11669							
809	59.2	74.5	10.0	0.36	8.9	51.9	3	705	3	602	—	—
810	60.9	73.8	11.7	0.33	10.6	54.9	3	773	3	673	—	—
811	60.9	73.9	13.7	0.31	12.3	55.4	2½	847	2¾	728	—	—
813	56.8	72.3	17.2	0.40	15.9	59.7	2½	1128	2½	1118	—	—
					COMANCHE CI 11673							
820	59.5	73.0	10.8	0.41	10.1	56.3	3½	755	4	660	—	—
821	58.9	76.5	12.7	0.43	11.8	59.2	4	855	4	795	—	—
822	56.9	72.0	15.7	0.45	14.7	63.1	3¼	1007	2¾	955	—	—
					NEBRED CI 10094							
818	60.3	71.4	14.4	0.38	13.0	59.8	3½	930	2¾	828	—	—
819	57.3	71.0	16.1	0.43	15.2	62.4	3¾	1062	3¼	985	—	—
					KAWVALE CI 8180 (SEMI-HARD, SOFT RED WINTER)							
804	58.1	73.6	9.5	0.40	8.3	52.6	3½	650	3½	560	—	—
805	58.3	73.2	10.4	0.39	8.9	52.5	3¼	700	3¼	597	—	—
806	60.0	72.0	11.7	0.38	10.4	54.7	3¼	808	3	692	—	—
807	57.7	73.7	13.0	0.39	11.7	57.3	2¾	857	2¾	740	—	—
808	57.8	71.0	14.9	0.38	13.3	58.1	2¾	1028	2¾	928	—	—
					MICHIGAN WONDER CI 5620 (SOFT RED WINTER)							
814	56.3	65.2	9.4	0.37	8.3	48.2	3½	647	2¾	532	—	—
815	57.7	65.0	10.2	0.36	8.7	49.1	3¼	693	2½	583	—	—
816	59.2	68.4	10.6	0.36	9.0	50.3	3½	707	2½	588	—	—
817	56.9	64.4	12.9	0.34	10.8	52.3	3	780	2¼	715	—	—
					CLARKAN CI 8858 (SOFT RED WINTER)							
786	61.0	65.2	9.7	0.32	8.3	52.3	2½	683	1¾	538	—	—
787	60.5	67.0	10.2	0.31	8.7	53.2	2½	725	1¾	570	—	—
788	60.9	66.8	11.4	0.31	9.9	54.6	2¼	768	1¾	638	—	—
789	60.9	66.8	12.8	0.32	11.3	55.2	2	798	1½	695	—	—
790	60.8	65.0	14.6	0.35	12.8	55.3	1⅞	812	1½	700	—	—

[1] Cleaned weights.
[2] On "as received" basis, not corrected to uniform moisture basis.
[3] Moisture basis 15%.
[4] Absorption used for Formulas 2 and 3, Formulas 1 and 4 values 4% higher.
[5] Mixing times: Formulas 1 and 4 the same, 2 and 3 the same.

rebaked using either 2 or 3 mg of bromate in the rich formula, it was found that this formula still consistently placed Blackhull ahead of Tenmarq. The modified MPB formula indicates that Blackhull is equal to Turkey and much superior to Tenmarq. The MPB formula, of the four formulas employed, placed Tenmarq the closest to the

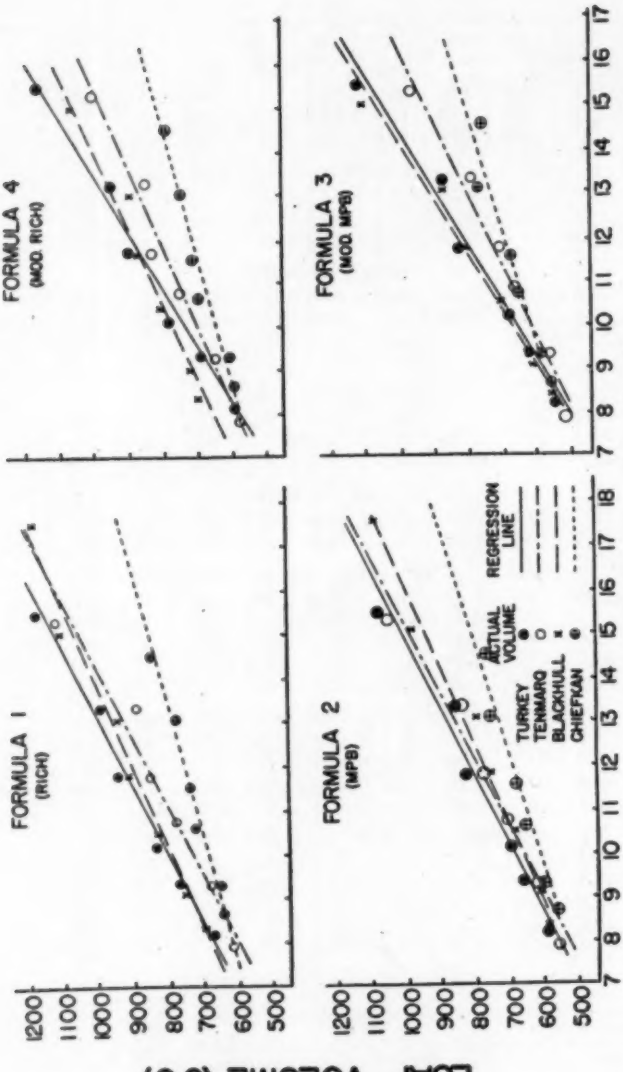

Fig. 1. The effect of four formulas upon loaf volume-protein regression lines of four widely different quality varieties.

equally desirable variety Turkey. Unfortunately this formula has the disadvantage of a relatively small spread between varieties. While spread between varieties is highly desirable, correctness of ranking is of paramount importance.

The baking data from these four varieties were sufficiently promising to warrant a comparison of the MPB formula with the rich formula on the remainder of the varieties. Figure 2 shows the regression lines

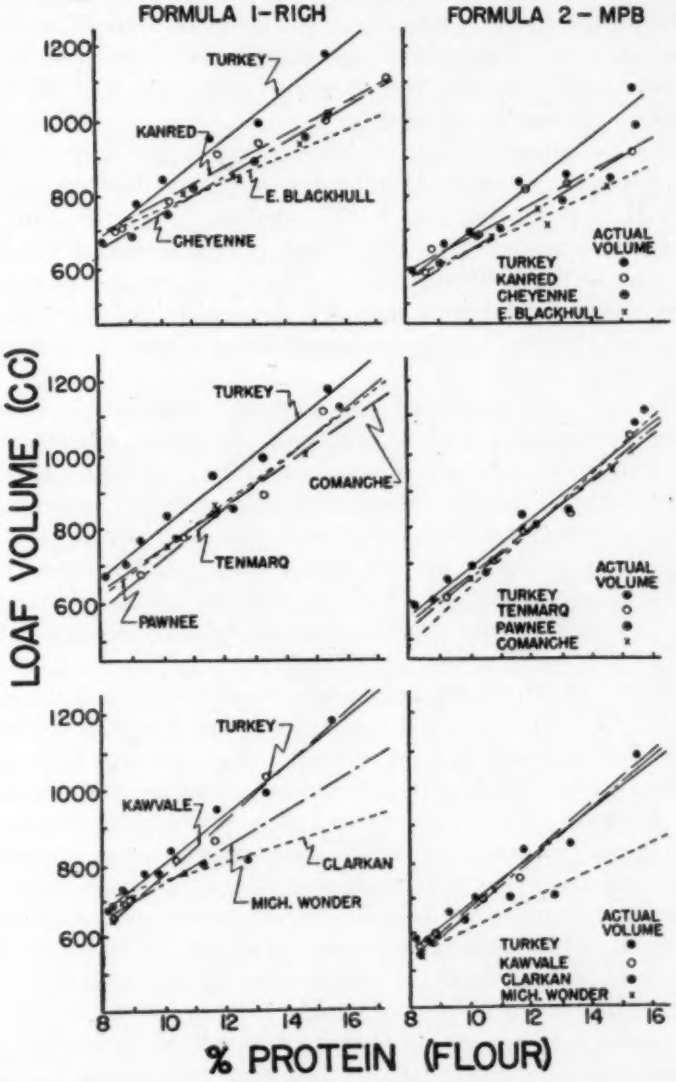

Fig. 2. A comparison of loaf volume-protein regression lines obtained from Turkey and several other varieties.

calculated from loaf volume and protein data for the remainder of the 14 varieties. Turkey has also been included for purposes of comparison. As only two protein levels were available for Nebred, no regression line is shown for this variety. The actual baking results with this variety show that with the rich formula Nebred possessed no particular advantage over Tenmarq of approximately equivalent protein content, while with the MPB formula the former was, if anything, inferior to the latter variety. At these protein levels Turkey gave larger volumes than Tenmarq with either formula. The behavior of these two Nebred samples seems to confirm the earlier results of West and Bayfield (1942), who concluded that Nebred needed milk in the formula to bring out its potential baking strength.

Among the minor acreage varieties grown in the hard red winter section of Kansas, Early Blackhull, Cheyenne, and Chiefkan are shown in Table II as possessing poor baking qualities. Kanred, which now occupies only a relatively small acreage, possessed only a fair baking performance in the 1940 crop tests. Additional experience is required with the newer hard winter varieties, Iobred, Iobred Selection, Comanche, and Pawnee, but in this particular year they gave promising baking results.

Among the varieties grading soft red winter, considerable differences in baking performance may be observed. Kawvale, which *looks* like soft red winter wheat, is seen to possess excellent baking quality, ranking with Turkey. As such baking performance is not desired in a soft red winter variety, this indicates *poor* quality in this variety as a *soft* wheat. The remaining two soft winter varieties, Michigan Wonder and Clarkan, are apparently distinctly different in the baking characteristics. This difference was noted by Bayfield, Working, and Harris (1941) in reporting upon the 1939 samples.

A number of interesting points may be observed from the data in Table II. In a general way it may be observed that loaf volume, absorption, flour ash, and flour protein tend also to increase with increasing protein content of the wheat, whereas test weight and mixing time tend to decrease. Flour yield shows a slight tendency to decrease with increasing protein content. While micro-mixer curves are not presented in this paper they were made on all the samples and these curves indicated shorter times to the peak of development and greater heights to the peak as the amount of protein increased. The curves bore a strong resemblance to those published earlier for the 1939 crop samples.

Comparison of the individual varieties shows definite differences in some respects. It may be noted that Blackhull, Early Blackhull, and Clarkan apparently give relatively less flour for a given test weight than varieties such as Turkey, Tenmarq, or Cheyenne. The former

three varieties yield low-ash flours. The high-test-weight variety Chiefkan is a relatively better flour-yielding sort than Blackhull. Iobred apparently is a low-flour-ash variety, whether the protein content is high or low. It is evident that milling qualities must be considered separately from baking characteristics. A variety may possess merit from a milling standpoint and be poor from a baking standpoint, or the reverse. A desirable variety needs to possess merit for both the miller and the baker.

The Regression Coefficient

The regression coefficients for loaf volume on flour protein were calculated for the different varieties when baked by the different formulas. The resulting information was used in preparing the regression lines shown in Figures 1 and 2. Except for the variety Clarkan, examination of the loaf volume and protein data indicate that these two factors are in an essentially linear relation.

McCalla (1940), working with spring wheats, has indicated that the loaf volume-protein content regression coefficient is a varietal characteristic which is inherited. He used a baking procedure adapted to wheats grown in Western Canada. Using Formula 4 with both a fixed and an optimum mixing time Bayfield, Working, and Harris (1941) found that the regression coefficient varied with varieties of hard winter wheat. Furthermore the coefficient was changed somewhat depending upon the mixing procedure used.

In the present study, using optimum mixing times, the formula has been varied, and, as shown in Figures 1 and 2, this change in formula frequently changed the slope of the regression line. It seems likely that, had further changes been made in formula, additional changes in the regression lines would also have resulted. As long as the regression lines are essentially parallel, no change in relative ranking of varieties will result. Unfortunately these lines frequently are not parallel for all varieties, and this is due it would seem to differences between varieties in response to baking ingredients or baking manipulations.

Barmore, Finney, and McCluggage (1941), working with hard red winter varieties, employed a formula similar to Formula 1 but with 2% less dry milk solids and sufficient bromate to give maximum loaf volume. They have employed the loaf volume-protein regression in computing a protein-quality correction factor. This procedure in the hands of these workers showed the variety Nebred to be exceptionally good as compared to the other varieties included in their study. Their method appeared to rank other commonly grown hard red winter wheats approximately as accepted commercially. The use of the optimum bromate level in the evaluation of new varieties introduces

a serious handicap. This is due to the frequent scarcity of flour and to the necessity for making a series of tests to determine the optimum bromate level for each sample.

Figures 1 and 2 show that the slope and relative height of the regression line may vary with the formula used. If a formula adapted to a good standard variety is used upon a series of varieties, it would seem that those varieties which gave regression coefficients and regression lines similar to that of the standard variety would also fit the market requirements met by the standard variety. On the other hand, varieties which gave regression lines much different from the standard when baked with the standard formula might be expected to cause trouble for the miller and baker. Similar trouble could be expected from varieties which gave regression lines similar to the standard variety only after given some special formula or other treatment. Such special treatments might include unusually short or long mixing times, special flour bleaching treatments, extra long or short fermentation times, or unusual requirements (in terms of the accepted standard) in any of the baking ingredients.

Summary

From a study of several hard and soft winter wheat varieties grown in Kansas in 1940 it is shown that choice of baking formula plays an important part in deciding which variety is most likely to suit the market requirements.

The possible use of the regression coefficient of loaf volume upon protein content as a protein-quality factor is discussed and it is shown that the type of regression line is dependent upon baking formula as well as upon variety.

It is concluded that in the *variety testing* program it is preferable to test new varieties by a method found to be adapted to a variety which is widely acceptable industrially. Those new varieties which do not respond in a manner similar to that of the standard variety are likely to prove unacceptable to the milling and baking industries even if they do possess otherwise potentially desirable characteristics.

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THE EFFECT OF HEAT TREATMENT OF MILK IN RELATION TO BAKING QUALITY AS SHOWN BY POLAROGRAPH AND FARINOGRAPH STUDIES [1]

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It is well known that fresh milk and milk products such as condensed or dried milk which have not been subjected to a sufficient heat treatment have poorer bread-baking qualities than properly heated milk. Numerous theories have been advanced as explanations for this effect of the heat treatment, such as the destruction of proteolytic enzymes, changes in the colloidal properties of the salts, denaturation and coagulation of the proteins, and changes in the oxidation-reduction systems.

Investigations in this laboratory indicate that the presence of sulfhydryl groups in unheated milk appears to be the major cause for its dough-softening or degrading action. Very small amounts of cysteine or glutathione will produce a rapid degradation of a dough. This action has been explained as due to the activation of proteolytic

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enzymes present in flour and also as due to a direct action on some flour constituent. The latter appears quite probable since the action of proteolytic enzymes in quantities sufficient to produce a poor-quality loaf of bread is slow, while equivalent quantities of certain sulfhydryl compounds produce a rapid dough-softening action readily observed during mixing. Such observations were also made by Balls and Hale (1936), Ford and Maiden (1938), and Freilich and Frey (1939).

Baerstein (1936) reported the sulfur content of milk proteins and the distribution as methionine and cystine as follows:

Protein	Percent sulfur	Percent of total sulfur as:	
		Cystine	Methionine
Casein	0.80	9.8	83.4
Lactalbumin	1.45	62.1	34.4

This indicates that milk proteins are potentially high in organic groups containing sulfhydryl groups.

Brdicka (1933) used the polarograph for the determination of cystine or cysteine in a buffered solution containing bivalent cobalt and found a current-voltage maximum at 1.5 volts. A current voltage curve is thus obtained which is characteristic of the sulfhydryl group or the disulfide linkage in cystine, the latter being first reduced to the sulfhydryl group at the mercury cathode. It is considered that the cobalt salt forms a complex with the sulfhydryl compound and that the characteristic current-voltage curve depends upon the catalytic reduction of the hydrogen of the sulfhydryl group.

Smith and Rodden (1936) found that methionine showed no reduction wave typical of cysteine or cystine. Mohr and Wellm (1936) prepared different protein fractions from milk and made polarographic analyses in the presence of hexamine-cobaltic chloride, which was used in the study of the protein effect. Somewhat different curves were obtained for various fractions, but they did not attempt to interpret these differences. They did not use cobaltous chloride, described by Brdicka as specific for the detection of sulfhydryl compounds.

In this study, polarographic analyses were made of heated and unheated fresh separated milk samples together with tests in the farinograph.

Experimental

A series of tests was made on milk using the Heyrovsky micropolarograph. The instrument is an automatic device designed to provide a photographic record of the current voltage curves in dropping mercury cathode analysis. A regularly increasing voltage is applied to the electrolytic cell, and the change in current flowing through the cell, as detected by a highly sensitive galvanometer, is automatically recorded on photographic paper as a function of the applied voltage.

The milk used in all of the tests was unpasteurized separated milk which was not over 30 hours old, and when reference is made to heated milk the heat treatment involved was holding the milk at 97°–100°C for five minutes. Several tests were made on milk from different lots.

A series of tests made with different final milk concentrations indicated that 0.4% in the final reaction mixture was an effective concentration to use. The curves obtained with 0.8% concentration were also easily reproduced, but the lower concentration was found to be better as a result of the high sensitivity of the instrument.

For polarograms the following solutions were quickly mixed in a test tube:

1.0 ml milk diluted 1–25 with water, or 1 ml 0.0001*M* cystine for the standard,
0.5 ml 0.05*M* cobaltous chloride,
2.0 ml buffer, 0.5*N* NH_4Cl and 0.5*N* NH_3,
6.5 ml distilled water.

This makes the final dilution of the milk 1–250 or 0.4%. The mixture was immediately introduced into the electrolytic cell and the measurements started. The solution must be used at once since standing causes alkaline decomposition of the milk proteins.

The results obtained with four samples of milk from different supplies are shown by the curves reproduced in Figure 1. The top curve was obtained by using 1 ml of 10^{-4} *M* cystine solution. The four curves to the left are from the heated milk samples and to the right are the corresponding curves of the raw unheated milk samples. It is clearly noticeable that with the unheated milk a maximum (at point A) occurs which is not present after heat treatment. Since, according to Brdicka (1933), this maximum is specific for organic sulfhydryl compounds, such as cysteine, in the presence of cobaltous chloride, it appears that heat treatment eliminates such groups present or exposed in the raw milk.

Three typical curves for cystine, raw milk, and heated milk are plotted in Figure 2, showing the galvanometer deflection at the calculated applied voltage. The maximum for the cystine at 1.5 volts is in agreement with Brdicka, while the maximum for the raw-milk curve is at approximately 1.43 volts. This might be due to the sulfhydryl groups in the milk being attached to the protein units and hence showing a maximum at a slightly different voltage than for free cysteine. In the case of the raw-milk curves a slight maximum was also observed at about 1.2 volts.

Figure 3 shows how the curves can be used to estimate quantitatively the concentration of the sulfhydryl groups as compared with the cystine curve from a known concentration. The minimum in the curve just past the maximum is measured. The first maximum noticed in all curves is due to the reduction of the cobaltous chloride at

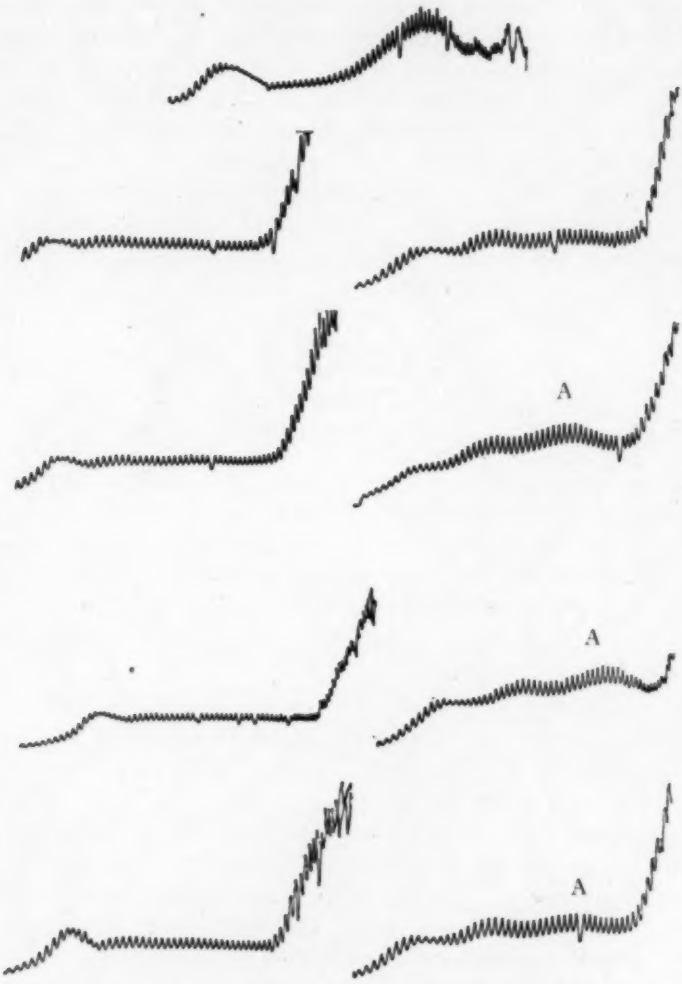

Fig. 1. The top curve is from cystine. The curves to the left are from heated milk samples, and to the right are the corresponding curves from unheated milk samples.

the lower voltage. The vertical distance between the two lines is a function of the difference in the concentration of the sulfhydryl groups.

The data in Table I give the calculated values of the sulfhydryl groups in the milk samples in terms of cysteine in the quantity of milk used for each curve—that is, 1 ml of milk diluted 1 to 25. They are also given in equivalents of cysteine-hydrochloride in 210 ml of milk, which is the approximate quantity of milk used with 300 g of flour for the mixing of a dough in the farinograph. The reactive organic sulfhydryl groups apparently varied appreciably with each raw-milk

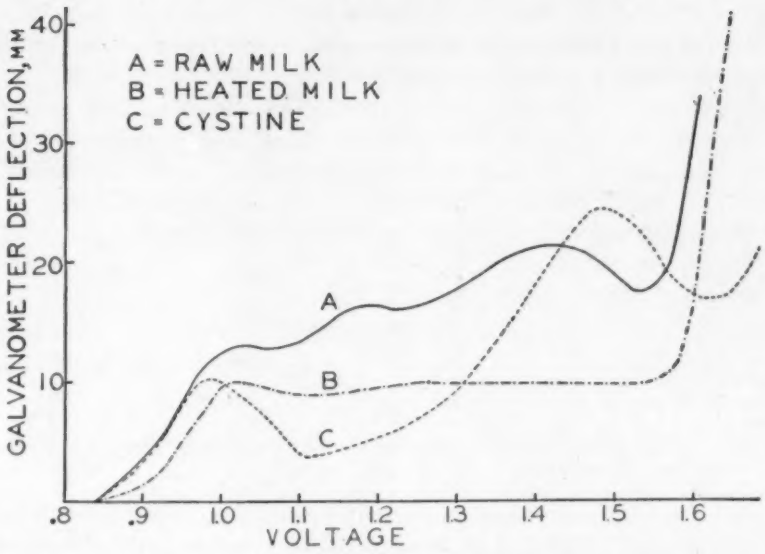

Fig. 2. Three typical curves showing the galvanometer deflection and the applied voltage.

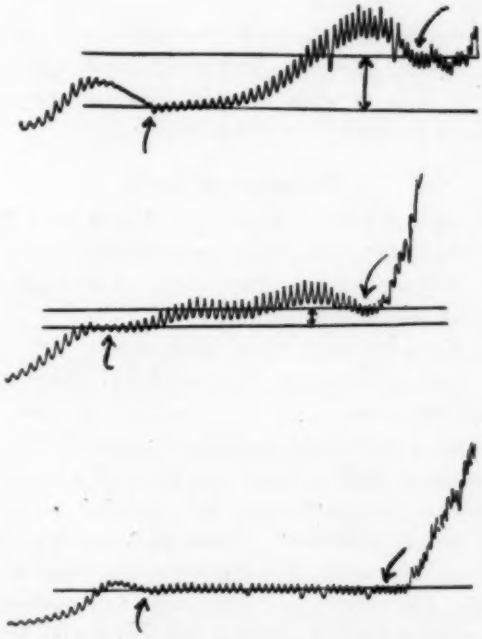

Fig. 3. This shows how the minimum just past the maximum was used for quantitative estimations. The top curve was obtained with a cystine solution of known concentration (final dilution $10^{-6}M$), the middle curve from unheated milk, and the lower curve from the same milk after a heat treatment.

TABLE I

CALCULATED QUANTITATIVE RELATIONSHIPS OF THE POLAROGRAPH CURVES

Milk used	Height of curve	Amount of reactive cysteine under the conditions of the polarograph test in milk used for test	Approximate equivalent of cysteine-hydrochloride in 210 ml milk
	mm	mg	mg
(1 ml $10^{-4}M$ cystine)	12	0.024	—
Boiled No. 1	0	*	*
Raw No. 1	4	0.008	52
Boiled No. 2	0	*	*
Raw No. 2	3	0.006	39
Boiled No. 3	0	*	*
Raw No. 3	5	.010	65
Boiled No. 4	0	*	*
Raw No. 4	4.5	.009	59
Average	—	—	52

* Quantities approaching 0.

sample, but all contained a sufficient quantity to cause a degradation of the dough. Addition of 39 to 65 mg of cysteine-hydrochloride to a 300-g flour dough would produce a rapid degradation of the dough during the mixing process. The boiled milk had no measurable amount of sulfhydryl groups.

These studies seem to indicate the necessity of heating milk properly for improved baking quality, and that elimination of the dough-softening action is a process of oxidation or occlusion of exposed sulfhydryl groups present in the raw milk.

Farinograph Tests

The farinograph or other recording dough mixers are recognized as being useful in the study of the baking quality of milk. A poor-quality milk will tend to give a farinogram showing a rapid dough softening after the point of maximum plasticity.

Using a medium-strength flour with about 11% protein and 2% salt (flour basis) in each dough, a series of farinograms was made with fresh milk and the whey and curd separated from the same milk sample by means of both acid and rennet coagulation.

A fresh separated milk sample was allowed to sour by standing at room temperature for 30 hours, and another sample was rennet-coagulated by using sufficient rennet to coagulate the milk in one hour at room temperature. The curd and the whey were separated by centrifugation. The whey was then given a five-minute heat treatment at 97°–100°C, cooled to 30°C, and recombined with the curd, and this preparation used for a farinogram with the described flour. Other farinograms were made similarly using the fresh milk, the coagulated milk, and the milk heated to 97°–100°C for five minutes.

The data in Table II give the results of these tests. The farinograms were analyzed as to time to reach minimum mobility, the maximum mobility, and the summation value. The summation value is the total value of all the breakdown units of the curves from the peak to the 30-minute stage using the units at every even-numbered minute. The actual curves are reproduced in Figure 4.

It is evident that the factor responsible for the dough softening action in unheated milk is present primarily and probably entirely in the whey portion. The curd undoubtedly retained some adsorbed whey, which accounts for the slight difference between the curves from heated milk and heated whey. Souring or coagulation alone did not materially change the curves.

TABLE II

RESULTS OF FARINOGRAPH TESTS USING A STANDARD FLOUR AND VARIOUSLY TREATED MILK PREPARATIONS

Curve no.	Material	Heat treatment	Time of minimum mobility	Minimum mobility	Summation value
		°C	*min*	*FU*	*FU*
1	Fresh separated milk	None	9.5	520	1350
2	Same milk rennet coagulated	None	11.0	495	885
3	Rennet coagulated milk, whey heated	97°–100° 5 min	13.0	485	510
4	Fresh separated milk	97°–100° 5 min	16.0	495	250
5	Fresh separated milk	None	10.5	520	1290
6	Soured milk, 30 hrs. room temp.	None	10.0	500	1105
7	Soured milk, whey heated	97°–100° 5 min	13.0	490	315
8	Fresh separated milk	97°–100° 5 min	17.0	490	105

Since the rennet whey gave the same results as the acid whey, it appears likely that calcium is not a factor involved. In the case of rennet coagulation, practically all of the calcium remains with the curd, but with acid-coagulated milk the calcium is found in the whey portion.

Several other tests including the use of hydrochloric-acid-coagulated milk also gave similar results, indicating that the dough-softening factor was present in the whey portion of the milk.

Discussion

The polarograms of raw milk have characteristics in common with polarograms of cysteine-cystine solutions. These characteristics disappear upon heat treatment. Considered as cysteine the quantity of such groups in raw unheated milk appeared more than sufficient to

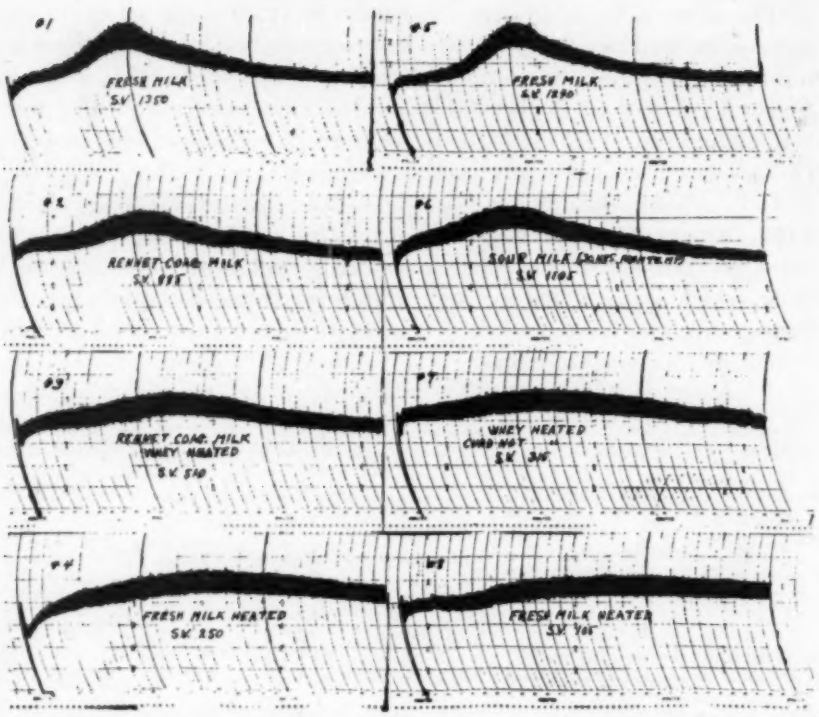

Fig. 4. Farinograms using fresh unheated milk (No. 1 and No. 5), coagulated milk (No. 2 and No. 6), coagulated milk in which the whey only was heat-treated (No. 3 and No. 7), and the fresh milk heat-treated (No. 4 and No. 8).

cause a softening action in wheat-flour doughs made with milk. The farinograph tests showed that the factor causing the dough softening was present in the whey portion of the milk. It is probably the lactalbumin which is involved, although the work of Sørensen and Sørensen (1939) indicates that the whey proteins are a complex mixture of several fractions. Computed from polarograms of the unheated milk samples the equivalent of 52 mg of cysteine hydrochloride in 210 ml of milk was present, which represents only about one-tenth of the sulfur in lactalbumin or about 15 hundredths of the total sulfur content of milk. Hence only a small portion of the total sulfur appears to be present as exposed or reactive groups in unheated milk under the conditions of this test.

While both the casein and the lactalbumin contain cystine sulfur, the concentration is generally reported to be much higher in the lactalbumin. Casein is present in milk in about ten times the amount of lactalbumin, yet it appears that the higher-molecular-weight casein proteins have few if any exposed or reactive sulfhydryl groups.

Sjögren and Svedberg (1930) after a study of milk with the ultra-

centrifuge concluded that lactalbumin as it occurs in raw milk is much more dispersed than any of the isolated products and that perhaps lactalbumin is not homogeneous. Their studies gave the molecular weights of 12,000 to 25,000 for the isolated lactalbumin, but they state that in the raw milk the bulk of the lactalbumin probably does not exceed a molecular weight of 1,000. Some authors (Hotchkiss, 1939) object to this rather low figure, but it is generally agreed that the molecular weight of lactalbumin is much lower than that of many other proteins. Ansbacher and Supplee (1934) and Supplee (1940) have shown that lactalbumin contains prosthetically bound lipid material in a fairly constant concentration of 7%, of which about 40% is cholesterol.

Some further experiments have shown that the dough-softening principle in unheated milk or whey dialyzes through Visking casing, as shown by farinograph tests only, but no direct tests for sulfhydryl groups were made. This indicates that a low-molecular-weight substance is involved. When the diffusate was heated a turbidity appeared indicating the presence of the heat-coagulable proteins.

While the experiments included in this report indicate that the heat treatment ordinarily given milk for the improvement of baking quality causes a decrease in groups reacting like cysteine, it is well known that denaturation or heating of egg albumin causes a marked increase in free sulfhydryl groups (Bull, 1938; Mirsky, 1938). However, a more severe heat treatment of milk or whey may cause a denaturation with a similar liberation of sulfhydryl groups. In fact Grewe and Holm (1928) and Skovholt and Bailey (1930) found that heating of milk at about 75° gave the best baking results, while higher temperatures or prolonged heating were less desirable.

Several workers (Gould and Sommer, 1939; Josephson and Doan, 1939) have observed that minute traces of hydrogen sulfide are formed when milk is heated and these cause the cooked flavor. Such small amounts of hydrogen sulfide probably would have no appreciable action in a milk dough and would perhaps be partially eliminated through aeration during the mixing process, but such quantities of hydrogen sulfide are still sufficient for organoleptic detection.

It appears that the following theory might account for the dough-softening action of unheated milk and the effect of the proper heat treatment: Fresh raw milk contains low-molecular-weight whey proteins high in sulfur, and these apparently are the proteins generally referred to as lactalbumin. These small protein molecules have some exposed or reactive sulfhydryl groups representing only a small part of the total sulfur. Upon proper heat treatment these proteins aggregate into particles of higher molecular weights. An oxidation of the sulfhydryl groups to the more stable disulfide linkage may take place,

or the sulfhydryl groups may be masked by occlusion. During this process, minute traces of sulfhydryl groups are released from the protein, forming free hydrogen sulfide, which may be of no material significance in terms of baking quality or dough-softening action but which causes the cooked flavor.

The heat treatment of milk necessary for the improvement of the baking quality appears to be analogous to the effect of the heat treatment of wheat. Sullivan, Howe, and Schmaltz (1937) showed that at least part of the beneficial effect of the heat treatment of wheat was the oxidation of the glutathione in the germ portion.

In this report the dough-softening factor in unheated milk has been discussed, but actually the baking quality of milk involves other factors also and particularly when evaporated or dried milk products are considered. Such other factors as absorption capacity, color, and flavor are also included in the general term of baking quality of milk products, but the dough-softening action of improperly heat-treated milk is one of primary importance in relation to baking quality.

Summary

It is known that a heat treatment is necessary to improve the baking quality of milk. Several samples of fresh unheated separated milk were subjected to polarographic analyses and each sample gave a current-voltage curve analogous to those obtained with cysteine-cystine solutions. When the same samples were heated for five minutes at 97°–100°C the polarograms no longer had this characteristic.

Quantitative estimations of the sulfhydryl groups in the raw-milk samples showed them to be present in amounts sufficient to cause a pronounced dough-softening action as compared to the action of cysteine hydrochloride.

After separating the curd and the whey of milk, it was shown by means of farinograms that the dough-softening factor in unheated fresh milk was present in the whey portion, and probably in the lactalbumin.

An hypothesis is advanced to explain the effect of heat treatment of raw milk in eliminating the dough-softening action. It is postulated that low-molecular-weight lactalbumin molecules with exposed sulfhydryl groups combine during the heat treatment to give protein aggregates of higher molecular weights. During this process the sulfhydryl groups are either oxidized to the more stable disulfide linkage, or masked by occlusion.

Acknowledgment

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A STATISTICAL STUDY OF THE DATA OF SUB-COMMITTEES ON TESTING BISCUIT AND CRACKER FLOURS

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The subcommittee of 1938–39 (Simmons, 1940) recommended:

1. That further work be done to build up a greater volume of data correlating the laboratory evaluation of cracker flours with their actual shop performance.

2. That further study be made on the scoring of crackers in order to obtain a closer agreement among collaborators.

3. That a statistical study be made of all pertinent data accumulated by the committees in the last three years, this to be done in order that more practical conclusions may be reached.

The first tool needed to correlate more closely the laboratory evaluation of cracker flours with their actual shop performance is a scoring method for crackers which will enable collaborators to agree as to which is the best cracker. The method of scoring should enable each collaborator to reach a definite numerical decision. Only then will it be possible to use the analytical data, the viscosity, and the baking tests to pick out a good sponge flour or a good dough flour.

Scoring Crackers

The present method of scoring makes it very difficult for a collaborator to recheck his decisions. It provides no mathematical score or measurable characteristics which can be scored in degrees of perfection.

Looking at Table V (Simmons 1940), one concludes that the first bakes of crackers P1, P2, P3, and P4 were so much alike that the collaborators were unable to agree as to just what position each cracker should be given. The second bake appeared to be slightly easier to score, but only P4 received the same rank as in the first bake. Surely the two bakes did not differ as much as the results show. Statistical correlation shows the marked inability of each collaborator to duplicate his results.

The scores for cracker P1, bake 1, were correlated with the scores for cracker P1, bake 2, using the results of eight collaborators. According to Wallace and Snedecor (1931) the value of the coefficient would have to be at least + .707 to be significant, and to be highly significant the coefficient should be + .834 or higher. The correlation

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coefficient for each cracker baked from Pacific coast flours shows that only the results given for cracker P3 are significant (Table I).

TABLE I

CORRELATION OF BAKES OF PACIFIC COAST FLOURS

Crackers	Coefficient of correlation
P1	+.10
P2	+.43
P3	+.74
P4	+.09

The same correlation study was made for crackers A, B, C, and D baked from midwest flours. Bakes 1 and 3 were used for this study; bake 2 was discarded because the crackers in this bake had an unusually high ash content (Table II).

TABLE II

CORRELATION OF BAKES OF MIDWEST FLOURS

Crackers	Coefficient of correlation
A	+.43
B	+.28
C	+.27
D	+.56

None of the scores for the midwest flours is significant.

In bakes 1 and 2 for the four crackers examined by the subcommittee of 1937–38 (Brown, 1939), the collaborators had much difficulty in correlating their results; in many cases negative correlation was shown (Table III).

TABLE III

CORRELATION OF CRACKERS BAKED IN 1937–38

Crackers	Coefficient of correlation
1	−.04
2	−.65
3	+.05
4	+.07

The method of scoring outlined by the Committee in their report of 1936–37 (Reiman, 1938) gives much importance to the shortness of the cracker. Forty out of the possible 100 points for a perfect cracker stress shortness in some degree, with 10 points for flakiness, 15 points for crispness, and 15 points for tenderness. If each collaborator is able

to score tenderness on this basis we should expect good correlation between the rank of the cracker and its shortometer rank (Table IV).

The correlation between rank and shortometer reading for midwest crackers, bakes 1 and 3, was + .41. The correlation between rank and shortometer reading for crackers in the 1937–38 report (bake 1, bake 2, and bake 3) was − .20.

Granting that an instrument such as a shortometer will give a correct numerical evaluation for shortness; and granting that flakiness, crispness, and tenderness scored by individual collaborators should reflect 40% of a total score for a cracker, we should expect—if the

TABLE IV

CORRELATION BETWEEN RANK AND SHORTOMETER READING FOR P CRACKERS, BAKE 1 AND BAKE 2

Crackers	Rank		Shortometer		
	ΣA	ΣA^2	ΣX	ΣX^2	ΣAX
Bake 1					
P1	1	1	86.6	7499.6	86.6
P2	2	4	83.7	7005.7	177.4
P3	3	9	87.3	7621.3	261.9
P4	4	16	87.6	7673.8	350.4
Bake 2					
P1	3	9	83.7	7005.7	251.1
P2	1	1	67.5	4556.3	67.5
P3	2	4	82.7	6842.3	165.4
P4	4	16	71.9	5169.6	287.6
	20	60	651.0	53374.3	1647.9

$MA = 2.5$
$MX = 81.3$

$$r = \frac{\Sigma AX - (\Sigma A)MX}{\sqrt{\Sigma A^2 - (\Sigma A)MA}\sqrt{\Sigma X^2 - (\Sigma X)MX}} = 0.33$$

collaborator is as accurate as the shortometer—a correlation coefficient of at least + .707. None of the above correlation coefficients is significant. The judging of the crackers of 1937–38 showed a negative correlation. The method of scoring used is obviously unreliable, for it depends upon the appraisals of collaborators whose judgments did not agree with the shortometer values.

The collaborator is unable to check himself on a value which involves 40% of the rating of an individual cracker. In addition 20% of the total rating represents personal reaction of the collaborator as to the flavor of the cracker. Flavor is a most delicate and difficult entity to characterize and systematically record.

The method of scoring allows 3% for symmetry of edge, 3% for shape, and 5% for salt; none of these has any relation to flours but to the fermentation and machining of the doughs. Therefore 71% of the total score is very difficult to check; it is no wonder that correlation between collaborators and bakes was not significant.

Correlation between Moisture and Shortometer Reading

As a cracker takes up moisture it becomes more soggy, less crisp, and less tender; hence one would expect the shortometer reading to be higher for a cracker with more moisture. In the 1938–39 report (Simmons, 1940), Table VII gives the moisture content and the shortometer values. Using the values in these two columns one obtains a correlation coefficient of + .203. To show a definite correlation between moisture and shortometer values the coefficient should be at least + .444.

Using the same type of data from the 1937–38 report (Brown, 1939) one obtains a correlation coefficient of + .79. To show a definite correlation between moisture and shortometer values the coefficient should be at least + .576, and the correlation is highly significant if it is + .708 or higher.

The moisture content of the cracker actually has some effect on the shortometer reading, but other factors are also important, as shown by the fact that the correlation for data from 1938–39 report is not significant.

Chemical and Physical Analysis of Crackers

Because all of the crackers should have been baked by the same formula, one would expect little difference in individual cracker analytical results. There is little difference in fat content if the results are figured on a dry basis. One would expect only small differences in flour protein and ash content, but for some unknown reason bake 4 (1938–39) gave crackers with exceptionally high ash and low protein content. Because of the abnormality in bake 4 none of its results were used in this study.

It is impossible to find any definite correlation between the score of a cracker and its protein, ash, or fat content. The crackers baked from the Pacific coast flours are the lighter crackers. The shortometer readings for the Pacific group are higher than those for the midwest group, but this can be expected because of the 1.5% lower fat content. It appears that chemical analyses of crackers, where all are baked by one formula, have little value for classifying flours used.

The count per pound and the average thickness of 10 crackers shows oven spring; and where flours of different strengths are used it is certain

TABLE V

CHEMICAL ANALYSIS OF CRACKERS (1938–39)

Cracker number	Protein, $N \times 6.25$	Ash	Fat	Shortometer value	Count per pound
	%	%	%		
P1	10.16	2.42	12.19	85.1	120
P2	10.20	3.03	12.40	75.6	122
P3	10.75	2.26	12.50	85.0	120
P4	10.80	2.80	12.53	79.8	120
A	9.18	2.76	14.43	76.3	111
B	9.03	2.53	14.33	75.3	116
C	9.07	2.67	14.09	81.2	111
D	8.88	2.95	14.01	70.0	112

that such data would be valuable. One other variable, the area, should be included in the physical analyses—namely, the flat area of four crackers. Such a measurement would reflect oven spread, which is presumably a measurement of strength.

Do Analytical Data Help to Indicate a Use for Flour?

Definite standings or ratings expressed as numbers are necessary to study correlation. For this reason a numerical rating is given in the column headed "Used For," Table I, 1938–39 report.

Weak dough	10
Medium dough	20
Strong dough	30
Weak sponge	40
Medium sponge	50
Strong sponge	60

In all the following correlation studies, the coefficient of correlation must be at least + .707; and to be highly significant it must be + .834 or higher.

Using the ash analyses as one variable and the numerical ratings from column 5, Table I, 1938–39 report, as the other variable, the coefficient of correlation was + .33. Ash analyses are not useful for indicating the utility of flours.

With pH values as one variable and the numerical ratings as the other, the coefficient is + .31; and thus the hydrogen ion concentration of the flour has no value in suggesting specific utility.

A correlation coefficient of + .793 was found when the protein values and the numerical ratings for the last four subcommittee reports were used. This figure is highly significant. The 1938–39 study gave a coefficient of + .92 for the Pacific flours, and a significant figure of + .98 was obtained for the midwest flours.

The protein content of a flour as a measure of its utility—if Column 5 of Table I, 1938–39 report is indicative of bake-shop use—is sta-

tistically important. If the designations in column 5 are merely millers' opinions, and do not reflect actual shop experience, it is evident that the opinions are based largely upon protein analysis.

Table II, 1938–39 report, gives average viscosity determinations using the one-hour and no-time methods. With one-hour values as one variable and the no-time values as the other, it was possible to get a correlation coefficient of 1.00. This is a perfect correlation, and it shows that the same factors present in the no-time method are equally important in the one-hour method.

Do Investigators Use the Viscosity Test to Classify Flours?

Table VI shows the numerical ratings given to data taken from Table III, 1938–39 report. To prove further that viscosity readings are very important in the final choice of the use of a flour, the average ratings of Table VI were correlated with the actual viscosity readings. The flours of the 1938–39 study, numbers 9 to 17, gave a correlation coefficient of + .92. Flours of the 1937–38 study gave a similar significant coefficient.

The viscosity test is very important to help classify flours, and all collaborators are using the viscosity readings to classify flours for use in the bakeshop.

TABLE VI

CLASSIFICATION ON BASIS OF ANALYTICAL AND VISCOSITY TESTS

Collaborator	Flour number							
	9	10	11	12	13	14	15	16
1	50	50	20	20	50	50	20	20
2	50	50	—	20	50	50	35	—
3	50	50	—	20	50	50	20	20
4	50	50	10	20	50	50	20	20
5	50	60	—		40	20	—	10
6	50	50	—	20	50	50	20	—
7	50	50	20	20	50	50	20	10
8	50	50		20	50	50	30	10
Average rating	50	51	17	20	49	46	24	15

Do Investigators Use the Baking Test to Classify Flours?

Classification on the basis of baking tests compares very closely with that based on viscosity tests. The 1938–39 report shows that flour 15, and the 1937–38 report shows that flour 8, are exceptions to the statement above. Unfortunately crackers were not baked from formulas using the two questionable flours as sponge flours. Every

flour to be tested should be tried as a sponge flour, for it is a well known fact that dough flours have little effect upon the finished cracker. It is interesting to note that crackers baked with flour 14 as the sponge flour and flour 15 as the dough flour gave a very inferior cracker. Perhaps the baking test will serve as a means of identifying the occasional flour that causes trouble in the bakeshop.

Table VII, showing a correlation coefficient of + .75, indicates that the baking test is serviceable in evaluating the use of flours, being almost equivalent to the viscosity test. Should the data for the abnormal flours 15 and 8 have been excluded, better correlation between baking test and viscosity test would have resulted.

TABLE VII

CORRELATION BETWEEN THE RATINGS BASED ON VISCOSITY AND BAKING TESTS

Year and flour	Evaluation by viscosity		Evaluation by baking		
1938	ΣA	ΣA^2	ΣX	ΣX^2	ΣAX
5	26	676	21	441	546
6	25	625	20	400	500
7	50	2500	39	1521	1950
8	18	324	40	1600	720
1939					
9	50	2500	46	2116	2300
10	51	2601	51	2601	2601
11	17	289	18	324	306
12	20	400	20	400	400
13	49	2401	49	2401	2401
14	46	2116	50	2500	2300
15	24	576	48	2304	1152
16	15	225	13	169	195
	391	15233	415	16777	15371

$MA = 32.6$ $MX = 34.6$ $r = +.75$

Summary

There is definite need of a scoring method for crackers which will enable collaborators to agree as to which is the best product.

The moisture content of a cracker has some effect on the shortometer reading, but other factors are also important.

Protein content and viscosity readings are statistically important as a measure of flour utility.

Classification of flours on the basis of baking tests agrees very closely with classification based on viscosity. The baking test may give additional means for the identification of flours that are likely to cause trouble in the bakeshop.

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THE RELATION BETWEEN THE VITAMIN-A-ACTIVE CAROTENOIDS IN CORN AND THE NUMBER OF GENES FOR YELLOW COLOR

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Yellow corn, unlike most plant materials, contains a large part of its vitamin-A-active pigments in the form of cryptoxanthin (Buxton, 1939; Fraps and Kemmerer, 1941; Kuhn and Grundmann, 1934) which, according to Kuhn and Grundmann (1933), has approximately one-half the vitamin A potency of beta-carotene.

Mangelsdorf and Fraps (1931) reported a linear relationship between the number of genes for yellow pigmentation in yellow corn, and in its hybrids with white, and the biological vitamin A potency of the corn as measured by the Sherman-Munsell method. Hauge (1930) and Hauge and Trost (1930) also have reported that there is a high degree of association between the number of genes for yellow color and the vitamin A potency. Johnson and Miller (1938, 1939) found a close relationship between the total carotenoid in yellow corn and the genetic constitution. Randolph and Hand (1938, 1940) found that doubling the number of chromosomes in yellow corn increased the total carotenoid pigments by approximately 40%. However, doubling the chromosomes also increased the volume of the endosperm cells, so that the actual amount of carotenoids elaborated per gene was approximately 2.5 times as great in the tetraploid as in the diploid. Randolph and Hand also found that the vitamin-A-active fractions, including both beta-carotene and cryptoxanthin, increased in approximately the same degree.

Recent investigations by Fraps and Kemmerer (1941) have shown that yellow corn contains, in addition to alpha-carotene, beta-carotene,

and cryptoxanthin, a carotene termed K-carotene. Methods for determining the quantities of these carotenes in yellow corn were developed, and it was shown that K-carotene, like alpha-carotene and cryptoxanthin, has only approximately one-half the vitamin A potency of beta-carotene.

With newer and improved methods of analysis available, it seemed desirable to ascertain the relationship between number of genes and amounts of carotenoid pigments contained in the crude carotene.

As in the previous work (Mangelsdorf and Fraps, 1931) appropriate pollinations were made to produce four classes of seeds, roughly described as white, pale yellow, dilute yellow, and deep yellow. In the case of the factor for yellow pigmentation, the cells of the endosperm have 0, 1, 2, or 3 genes for the yellow pigment, with the corresponding factorial composition, *yyy*, *yyY*, *yYY*, or *YYY*. Four varieties of white corn, Paymaster, Surecropper, Mexican June, and Thomas, and four varieties of yellow corn, Yellow Paymaster, Yellow Surecropper, Golden June, and Golden Thomas, were used. All pollinations were made between the yellow and white strains of the same variety. In the case of the last three, the yellow strains were developed from the white by hybridization and repeated backcrossing and, with the exception of the genes for color, were approximately isogenic with the original white varieties. The procedure followed in this experiment has thus eliminated variations in carotene content resulting from differences between white and yellow varieties in the size, shape, and texture of the grain.

Experimental

The samples of corn were finely ground, refluxed with alcoholic potassium hydroxide, and the crude carotene fraction obtained as in the A. O. A. C. method for carotene (A. O. A. C., 1940). This crude carotene fraction has been shown to contain all the vitamin-A-active constituents of yellow corn (Fraps and Kemmerer, 1941). The pigments in the crude carotene fraction were separated and identified by running the solutions through a column of magnesium oxide by a method reported also by Fraps and Kemmerer.

The results of this study are shown in Table I. Although there are some variations, the data show as has been reported in previous work (Mangelsdorf and Fraps, 1931) that the number of genes for yellow color in corn directly affects the amount of carotenoid pigments and the vitamin A potency. The corns of the genic constitution *YYY* contained on the average 6.4 ppm of crude carotene, the corn of *YYy* constitution 4.2, and the corns of *Yyy* constitution 1.8. The corn of *yyy* constitution contained only 0.1 ppm of crude carotene. The data

in this table also show that the number of genes for yellow color had no significant influence on the average percentage of alpha, beta, and K carotene and cryptoxanthin comprising the crude carotene.

There were some variations between the individual samples in the percentages of the carotenoids in the crude carotene. For example, all of the samples in which the genes for yellow color were derived from

TABLE I

EFFECT OF THE NUMBER OF GENES FOR YELLOW COLOR ON THE VITAMIN-A-ACTIVE PIGMENTS OF YELLOW CORN

Variety of corn	Crude carotene	Constituents of crude carotene				
		Alpha carotene	Beta carotene	K carotene	Cryptoxanthin total	Impurity
YYY group	*ppm*	%	%	%	%	%
Yellow Paymaster	3.9	4.8	33.2	6.4	52.6	0
Yellow Surecropper	6.6	3.5	36.0	5.5	52.2	2.9
Golden June	7.1	4.2	22.1	3.6	66.7	3.6
Golden Thomas	7.8	3.8	37.8	3.9	51.2	1.8
Average (4)	6.4	4.1	32.3	4.9	55.7	2.1
YYy Group						
Yellow Paymaster X White Paymaster	3.7	4.3	36.4	5.2	53.0	1.7
Yellow Surecropper X White Surecropper	3.6	6.6	27.6	3.3	58.6	3.8
Golden June X White June	3.9	8.1	18.3	4.1	65.6	4.1
Golden Thomas X White Thomas	5.5	4.4	32.0	3.3	58.2	2.6
Average (4)	4.2	5.9	28.6	4.0	58.9	3.1
Yyy Group						
White Paymaster X Yellow Paymaster	0.9	7.3	27.7	8.0	54.5	2.7
White Surecropper X Yellow Surecropper	1.1	—	—	—	49.9	6.4
White June X Golden June	2.5	4.0	19.8	5.9	57.3	12.9
White Thomas X Golden Thomas	2.5	3.3	37.5	4.2	50.8	5.0
Average	1.8(3)	4.9(3)	28.3(3)	6.0(4)	53.1(4)	6.8

Golden June were consistently higher in cryptoxanthin than were the remaining three samples. Fraps and Kemmerer (1941) have previously shown that corn may be divided into two rather distinct groups with respect to the constituents of the crude carotene. Whether or not variations of this kind were a consequence of differences in behavior of the *Y* gene on different genetical backgrounds, or the effect of various

modifying factors, was impossible to determine from this experiment. Nevertheless, it is clear that there is an almost perfect linear relationship between the genic constitution of the endosperm and the amount of crude carotene, and that this relationship holds approximately for each of the separate constituents of crude carotene.

Conclusion

The number of genes of yellow color in corn directly affects the amount of vitamin-A-active carotenoid pigment, but does not affect appreciably the relative proportions of the different constituents, alpha, beta, and K carotene and cryptoxanthin.

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THE THIAMIN CONTENT OF WHEAT FLOUR MILLED BY THE STONE MILLING PROCESS

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The modern roller mill, now almost universally employed for grinding wheat, has very little in common with the old stone mill. The roller mill grinds the wheat by a series of gradual reductions between carefully constructed and accurately spaced rolls; at no time is the wheat berry crushed or subjected to intense friction as it was in the mill-stone process. The difference in treatment has undoubtedly resulted in a different end product.

The introduction of roller mills occurred at a time when vitamins were quite unknown, and consequently we have no direct knowledge of the effect of the new process on the vitamin content of the flours then being produced. Recently Mitchener (1941), Miller (1941), and Rich (1941) have considered the changes which have occurred in the vitamin B_1 content of white flour produced by the old and new processes. Each concludes, reasoning by analogy, that the white flour produced one hundred years ago contained no more thiamin than the modern product.

According to these authors the ancients produced fine "white" flour equal to our modern flour. The evidence for this assumption is based on records which show that some of these old mills obtained a flour of only 55% to 70% extraction which they described as "white flour" or "superfine flour," etc. The analogy is completed by noting that the modern miller obtains the same low extraction and also calls his product "fine white flour." Obviously the adjective "white" as applied to flour was and still is a purely relative term.

Baker, Wright, and Drummond (1937) and Drummond and Wilbraham (1939) conclude that stone-ground white flour contained notably more thiamin than patent flour. Their contention is supported by analysis of stone-ground flour made in an English mill. Wheat containing 6.3 μg of thiamin per gram gave a flour containing 4.95 μg per gram. These authors emphasize the removal of germ by the roller mill but, as Schultz, Atkin, and Frey (1939) have shown, germ contains hardly more than 10% of the wheat thiamin. It seems more probable that stone-ground flour is richer in thiamin than patent flour because it retains a significant proportion of the aleurone layer of the wheat berry, which, if we may judge from the thiamin content of the

shorts of a modern mill stream, may contain more than 30% of the wheat thiamin.

Whatever the explanation of the thiamin distribution may be, it remains to be determined whether stone-ground white flours were relatively rich in thiamin or whether they were no better than modern patent flour, as Mitchener, Miller, and Rich claim. We have been fortunate in obtaining the cooperation of the managers of two American flour mills operating as closely as possible to the methods of a century ago. The mill streams of these mills were assayed by the fermentation method embodying the latest modification, sulfite cleavage (Schultz, Atkin, and Frey, 1942). The results are shown in Table I.

TABLE I

THIAMIN CONTENT OF STONE-MILL PRODUCTS[1]

Fraction	Mill G, thiamin	Extraction	Mill P, thiamin (August)	Extraction	Mill P, thiamin (September)	Extraction
	μg/g	%	μg/g	%	μg/g	%
Bran	5.82	—	9.8	—	9.18	—
Middlings	6.27	—	4.46	—	6.0	—
Flour	4.04	54	4.74	55	4.52	62
Wheat	5.2	—	4.67	—	5.6	—

[1] The results of further work indicate that the high thiamin content of stone-ground flour is accompanied by proportionately high concentrations of other vitamins of the B complex as well as of ash.

The flours of the three millings represent 54%, 55%, and 62% extraction, respectively, which compares favorably with the low extractions obtained in milling modern patent flours. Mill G produced a flour containing 4.04 μg of thiamin per gram, or 78% as much as the original wheat. Mill P in August produced a flour slightly richer than the original wheat, and in September produced a flour containing 4.52 μg of thiamin per gram, representing 80% of the original wheat concentration.

The stone-milled white flours were fine products, somewhat creamier in color than modern patent flour. When baked into bread they could not stand color comparison with modern white bread but each gave bread that was far whiter than whole-wheat bread and it required no stretch of the imagination to believe that they would have been called "fine white bread" a hundred years ago. The comparisons mentioned were graphically displayed on Kodachrome transparencies but such photographs are not suitable for reproduction.

It must be concluded, therefore, that stone-ground white flour made today in the best-remembered fashion of past centuries contained a concentration of thiamin roughly equivalent to the whole grain.

This does not mean that all of the thiamin of the wheat berry was consumed directly by man for, as Table I shows, the offal or feed by-products were equal in thiamin content to the flour. Today the feed produced is far richer in thiamin than the original wheat.

The per-capita consumption of thiamin must have been far greater in the days of the stone mill than today because, as we are informed, the per-capita consumption of bread was higher and the bread that was eaten was notably richer in thiamin.

Summary

White flours produced at the present time in two stone mills operating in the manner of the past century were examined for thiamin content and found to contain thiamin in approximately the same concentration as the original wheat.

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THE STABILITY OF VITAMIN B_1 IN THE MANUFACTURE OF BREAD

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The question of the stability of vitamin B_1 in the manufacture of bread gains increased importance as a result of the present national program for the enrichment of white bread. It is desirable to determine as far as possible the extent of vitamin B_1 loss in normal bakery practice so that the bread produced will meet whatever claims are made for it or whatever minimum standards may be set up. It is also desirable from the standpoint of economy to establish the conditions which result in minimum vitamin loss.

Recent reports (Hoffman, Schweitzer, and Dalby, 1940; Food and Drug Administration, Bread Hearings, 1941; Dawson and Martin, 1941) have indicated that the loss in thiamin during baking may vary from 5% to 30%. Part of the data of the present communication were presented at the bread hearings.

There are several factors which might conceivably affect the stability of thiamin during baking: the nature of the thiamin source, the time and temperature of baking, acidity, and the composition of the dough. We have found that the source of thiamin is without any significant effect; vitamin breads made with whole-wheat flour, white flour plus high-vitamin yeast, and white flour plus synthetic-thiamin all showed substantially the same destruction of thiamin when baked at the same temperature and for the same length of time. The time and temperature of baking appeared to have the greatest influence on the destruction of thiamin under our conditions and therefore several series of loaves were baked for different lengths of time but all at the same oven temperature.

Methods

The determination of thiamin was made by the yeast fermentation method, which included the sulfite modification (Schultz, Atkin, and Frey, 1942). In this method the thiamin content of the unknown is determined by the difference between two measurements of fermentation activity, one measurement before and one after treatment of the sample with sulfite. The first measurement determines the total fermentation stimulating substances (FSS) and the second determines the residual stimulators after the thiamin has been specifically inactivated by sulfite. The residual activity is called the "sulfite blank." The

thiamin content of the sample is represented by the difference between FSS and sulfite blank.

The sulfite blank reflects the presence of interfering aminopyrimidine fermentation activity, which is unaffected by sulfite treatment (Schultz, Atkin, Frey, and Williams, 1941). It has previously been observed and is also demonstrated in the present communication that the sulfite blank of a material is increased when its thiamin content is lowered as a consequence of excessive heat or alkalinity. The appearance of additional sulfite blank under these conditions is interpreted as being due to the formation of aminopyrimidines from thiamin, by a process of hydrolytic cleavage. The appearance of an unusually large sulfite blank is, therefore, an indication that thiamin has been destroyed by heat or alkali. The sulfite blank does not increase in exact proportion to the thiamin which has been destroyed, but one observes from 30% to 50% yield; *i.e.*, if 1.0 μg of thiamin has been destroyed the sulfite blank is increased by 0.3 to 0.5 μg.

Experimental

If the various ingredients of the dough batch are separately analyzed and a summation of these values considered as the vitamin input, it is possible that several small errors will be added together to give a result difficult to interpret. This is particularly true when one of the ingredients is patent flour, which is so low in thiamin content that most methods are least accurate with this material. It was preferred, therefore, to analyze the dough batch after mixing.

The laboratory bakes (Tables I to VI inclusive) were based on the straight dough process with the following formula (percentages expressed on the weight of flour): 2% salt, 5% cane sugar, 1% dry malt, 6% milk solids, ⅓% Arkady, and 2% yeast. For each experiment a dough batch containing 3,000 g of flour was mixed. This represented enough dough for 10 one-pound loaves of bread. The whole dough mass was weighed just before molding and one-tenth of the weight was scaled off for each of eight bakes. At the same time two weighed aliquots of the dough were taken for analysis. These were immediately macerated with water in a Waring Blendor, acidified, and heated at 100°C for 20 minutes. Two loaves of each of the doughs were baked for each of the following periods: 10, 20, 30, and 40 minutes—at 410°F in a gas-fired oven in which the loaves are rotated in a vertical plane.

The loaves were cooled for one hour at room temperature and then weighed. One loaf of each pair was prepared for total thiamin analysis by maceration in water, acidification, and heating at 100°C. The other loaf was dissected into three approximately equal portions: the crust with adhering crumb, the innermost crumb, and the intermediate

zone. Because of differences in moisture content it was necessary to air-dry these fractions and then determine the solids content of the air-dry samples. The thiamin content of the various zones was then compared on the dry-solids basis.

Enriched white bread (synthetic thiamin): A commercial enriching concentrate (containing synthetic thiamin) was thoroughly mixed to insure uniformity and then incorporated in a dough in the proportion of 1 g of concentrate to 300 g of flour. Table I gives the data obtained on the whole loaves of this series. In appearance the 10-minute loaf was definitely underbaked, whereas the 40-minute loaf was as definitely overbaked. A progressive loss of thiamin was observed as the time of baking was increased. At 10 minutes the destruction was very slight, almost within the experimental error of measurement, but at 20 minutes

TABLE I

EFFECT OF BAKING TIME ON B_1 CONTENT OF ENRICHED WHITE BREAD (SYNTHETIC THIAMIN)

Length of baking	Weight of loaf	Total FSS	Sulfite blank	Thiamin	Thiamin, average of two[1]	Loss of thiamin
min	*g*	*μg/loaf*	*μg/loaf*	*μg/loaf*	*μg/loaf*	*%*
0 (dough)	—	2219	173	2046	—	—
10	504	2140	186	1954	1975	3
20	485.5	2040	186	1854	1780	13
30	464.5	1890	275	1615	1620	21
40	448	1710	390	1320	1370	33

[1] Average of whole loaf and dissected loaf (see Table II).

the destruction was 13%. At 30 minutes the thiamin loss was 21% and since this loaf was a well-baked one, 21% should represent the maximum loss to be expected in normal bakery practice.

The magnitude of the sulfite blank was increased as thiamin was inactivated by increased baking time, which indicates that a portion of the thiamin is split as it is inactivated. The increase in sulfite blank was not equivalent to the thiamin destroyed but equaled only 29% in the 40-minute bake. An increase in sulfite blank, or an unusually high sulfite blank, indicates that thiamin destruction has occurred. If one determines only FSS (fermentation stimulating substances) without sulfite correction, erroneous conclusions may be reached. If the destruction of thiamin in the 40-minute bake (Table I) is calculated from the FSS figures, one obtains only 23% destruction compared with 33% based on thiamin content.

The analyses of crust and crumb fractions (Table II) indicate that the major part of thiamin destruction occurs in the crust. The thiamin content of the crust is reduced by 50% as the time of baking is extended

TABLE II

EFFECT OF BAKING TIME ON THIAMIN CONTENT OF CRUST AND CRUMB OF ENRICHED WHITE BREAD. (SYNTHETIC THIAMIN)

Time of baking	Weight of loaf	Weight of fraction, dry-solids basis[1]	FSS, dry-solids basis	Sulfite blank, dry-solids basis	Thiamin, dry-solids basis	Thiamin per loaf (calc)
min	*g*	*g*	*μg/g*	*μg/g*	*μg/g*	*μg*
10	498	O 106.7	7.18	0.66	6.52	2002
		M 97.5	7.43	0.74	6.69	
		I 99.9	7.17	0.62	6.55	
20	481	O 115.7	6.16	1.02	5.14	1705
		M 100.5	6.58	0.76	5.82	
		I 87.5	6.69	0.70	5.99	
30	465	O 107	5.61	1.21	4.40	1626
		M 98.8	6.89	0.95	5.94	
		I 96.98	6.68	0.83	5.85	
40	449	O 110	4.66	1.45	3.21	1422
		M 103.7	6.60	1.00	5.68	
		I 88.2	6.42	0.99	5.43	

[1] O signifies the third of loaf including the crust. M signifies the third of loaf between crust and innermost crumb. I signifies the third of loaf including the innermost crumb.

from 10 to 40 minutes, whereas in the same time the crumb has lost only 17% of its thiamin. The progressive increase in sulfite blank is also observed in this series, and as might have been expected the increase is greater in the crust where the greater thiamin destruction has occurred.

Whole-wheat bread: A series of loaves was baked with whole-wheat flour. The results (Tables III and IV) indicate that thiamin destruction occurs to the same general extent as in enriched white bread. It appears that the difference in origin of the thiamin has no noticeable effect upon its stability in the baking of bread.

Enriched white bread (high B_1 yeast): High vitamin B_1 white bread with yeast as the source of the vitamin was baked by substituting high B_1 yeast in the formula. The results of this series (Tables V and VI) are substantially the same as obtained with whole wheat bread and with white bread enriched with synthetic thiamin.

Bakery scale experiment: The laboratory-baked loaves were baked for fixed lengths of time—10, 20, 30, and 40 minutes—regardless of the fact that the loaf was underbaked at 10 and overbaked at 40 minutes. In order to compare the various types of bread under normal bakery conditions, 50-pound doughs were baked in a full-size electric bakery oven, being handled in the usual manner to produce normal loaves of bread. The sponge dough process was employed. Samples for analysis were taken from the dough stage, at which time the dough was

TABLE III

Effect of Baking Time on Thiamin Content of Whole-Wheat Bread

Time of baking	Weight of loaf	Total FSS	Sulfite blank	Thiamin	Thiamin, average of two[1]	Loss of thiamin
min	*g*	*μg/loaf*	*μg/loaf*	*μg/loaf*	*μg/loaf*	*%*
0 (dough)		1921	173	1748	—	—
10	517	1940	207	1733	1708	2
20	499	1872	364	1508	1446	17
30	480	1760	367	1393	1352	23
40	468.5	1632	444	1188	1167	33

[1] Average of whole loaf and dissected loaf (see Table IV).

TABLE IV

Effect of Baking Time on Thiamin Content of Crust and Crumb of Whole-Wheat Bread

Time of baking	Weight of loaf	Weight of fraction, dry-solids basis[1]	FSS, dry-solids basis	Sulfite blank, dry-solids basis	Thiamin, dry-solids basis	Thiamin per loaf (calc)
min	*g*	*g*	*μg/g*	*μg/g*	*μg/g*	*μg*
10	512.5	O 110	6.07	0.98	5.09	1682
		M 99	6.52	0.61	5.91	
		I 97	6.27	0.74	5.53	
20	496.5	O 117	5.38	1.17	4.21	1384
		M 90	5.61	1.09	4.52	
		I 99	5.89	1.03	4.86	
30	480	O 118	4.65	1.27	3.38	1312
		M 92	6.04	1.28	4.76	
		I 94	6.03	1.03	5.00	
40	466.5	O 125	4.28	1.58	2.7	1146
		M 93	5.84	1.39	4.45	
		I 87	5.92	1.39	4.53	

[1] O signifies the third of loaf including the crust. M signifies the third of loaf between crust and innermost crumb. I signifies the third of loaf including the innermost crumb.

TABLE V

Effect of Baking Time on Thiamin Content of Enriched White Bread (High B_1 Yeast)

Time of baking	Weight of loaf	Total FSS	Sulfite blank	Thiamin	Thiamin, average of two[1]	Loss of thiamin
min	*g*	*μg/loaf*	*μg/loaf*	*μg/loaf*	*μg/loaf*	*%*
0 (dough)		2375	183	2192		
10	498.5	2352	217	2135	2052	6
20	482	2248	320	1928	1877	14
30	461.5	2064	358	1706	1683	23
40	445.5	1968	396	1572	1503	32

[1] Average of whole loaf and dissected loaf (Table VI).

TABLE VI

EFFECT OF BAKING TIME ON THIAMIN CONTENT OF CRUST AND CRUMB OF ENRICHED WHITE BREAD (HIGH B_1 YEAST)

Time of baking	Weight of loaf	Weight of fraction, dry-solids basis[1]	FSS, dry-solids basis	Sulfite blank, dry-solids basis	Thiamin, dry-solids basis	Thiamin per loaf (calc)
min	*g*	*g*	*μg/g*	*μg/g*	*μg/g*	*μg/loaf*
10	495	O 95.6	7.33	1.21	6.12	1969
		M 99.7	7.84	1.08	6.76	
		I 108	7.55	0.97	6.58	
20	478.5	O 99.3	6.34	1.39	4.95	1826
		M 100	7.49	1.20	6.29	
		I 103.3	7.78	0.96	6.82	
30	462.5	O 104.4	5.49	1.40	4.09	1659
		M 102.1	7.27	1.16	6.11	
		I 967	7.29	1.02	6.27	
40	450.5	O 109.1	4.96	1.66	3.30	1434
		M 100.2	6.89	1.42	5.47	
		I 92.9	7.15	1.35	5.80	

[1] O signifies the third of loaf including the crust. M signifies the third of loaf between crust and innermost crumb. I signifies the third of loaf including the innermost crumb.

weighed. The yield of bread was also determined (one hour out of the oven) and the relation of dough to bread could then be calculated. The results are given in Table VII, from which it appears that destruc-

TABLE VII

STABILITY OF THIAMIN IN BREAD BAKED ON A COMMERCIAL SCALE

	Dough thiamin[1]	Bread thiamin	Thiamin loss
	μg/loaf	*μg/loaf*	%
White bread (high B_1 yeast)	1882	1469	22
White bread (synthetic thiamin)	2140	1687	21
Whole wheat bread	1887	1334	26

[1] Calculated on the basis of observed bread yield per unit weight of dough.

tion in these "normally" baked loaves approximated that observed in the laboratory bakes at 30 minutes.

Summary

The stability of thiamin in the baking of bread was not affected by the source of thiamin. Synthetic thiamin, high vitamin bakers' yeast, and whole wheat were the sources used.

The extent of thiamin destruction was markedly influenced by the time of baking. Loaves of "normal" bake showed a thiamin loss of approximately 20%.

The major portion of thiamin destruction occurred in the crust although destruction in the innermost crumb was not insignificant. Results with large-scale bakery experiments agreed with the laboratory results.

Acknowledgment

The laboratory bakes were made by Mr. Stanley A. McHugh, and the large-scale bakes by Mr. Harry Ekstedt. We wish to express our thanks to both of these men for their willing cooperation.

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BOOK REVIEW

Modern Bread from the Viewpoint of Nutrition. By Henry C. Sherman and Constance S. Pearson. 104 pages. Macmillan Company, New York. Price $1.75.

According to the jacket, "If it is your job to plan diets or to advise others in the management of a food budget with a view to keeping down cost without sacrificing the essentials of adequate nutrition, you will want this book." From the Columbia University Nutrition Laboratories comes this latest discussion of the place of bread in modern nutrition. The authors describe the importance of bread as a source of energy, proteins, minerals, and vitamins and discuss means by which these values may be enhanced. A discussion of the role to be played by enriched flour (written in August, 1941) and the value of the enriching compounds and elements is included. The improvement of nutritive values observable when dried milk is used in bread making is stressed. The authors point out that calcium might well be considered a valuable adjunct in addition to thiamin, nicotinic acid, and iron. They conclude that when flour is properly supplemented with vitamins and minerals by the addition of synthetic compounds, or when it contains vitamins and minerals by virtue of residual bran and germ, bread made therefrom with dried milk may logically be used to furnish as much as 40% of the total calories in the diet. There are 84 literature references.

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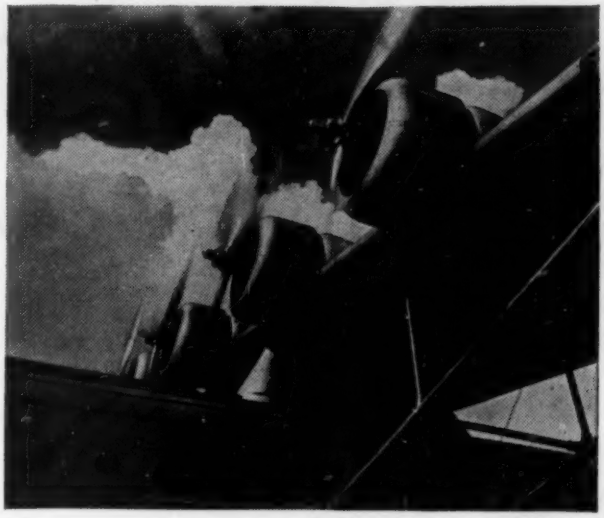

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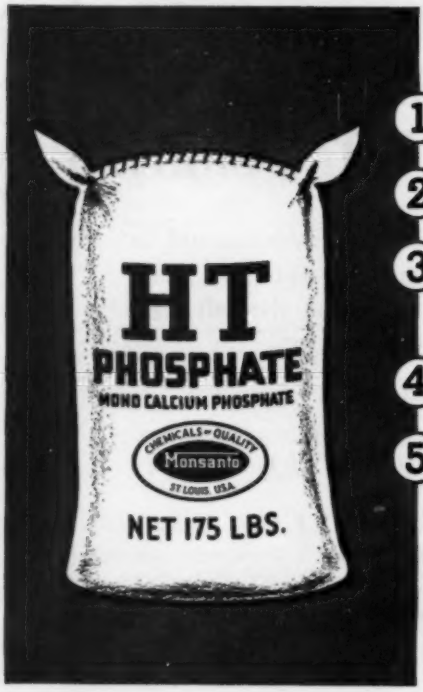

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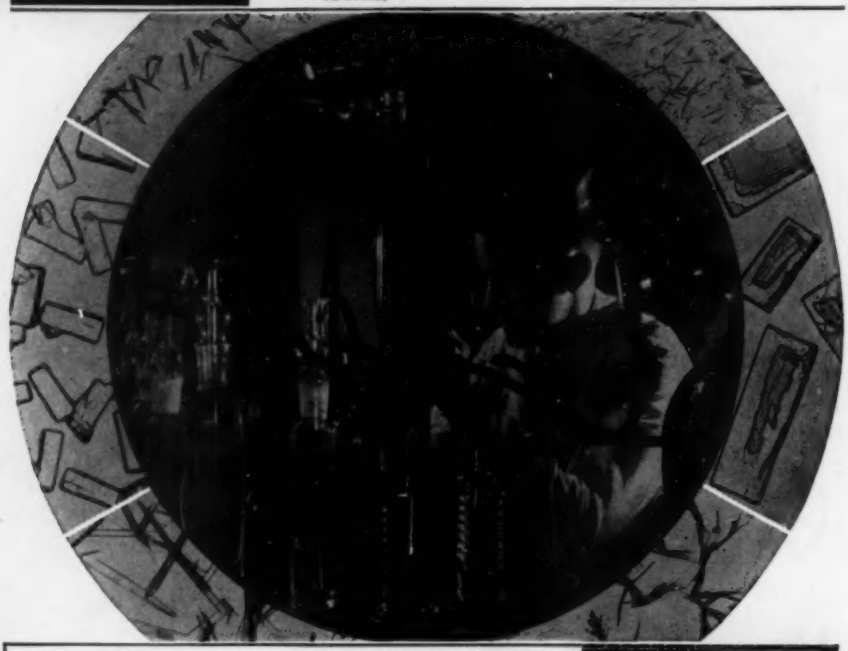

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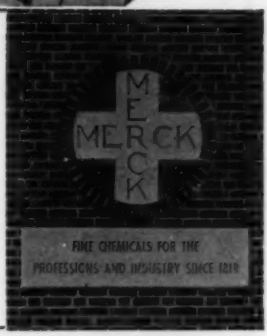

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